

Evaluation of the Tentative Standard Method for
Enteric Virus Concentration and Detection

North Carolina Univ. at Chapel Hill

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EVALUATION OF THE TENTATIVE STANDARD METHOD
FOR ENTERIC VIRUS CONCENTRATION AND DETECTION

by

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FOREWORD

The U. S. Environmental Protection was created because of increasing public and governmental concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our national environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The primary mission of the Health Effects Research Laboratory in Cincinnati (HERL) is to provide a sound health effects data base in support of the regulatory activities of the EPA. To this end, HERL conducts a research program to identify, characterize, and quantitate harmful effects of pollutants that may result from exposure to chemical, physical, or biological agents found in the environment. In addition to the valuable health information generated by these activities, new research techniques and methods are being developed that contribute to a better understanding of human biochemical and physiological functions, and how these functions are altered by low-level insults.

This report describes the results of an evaluation of tentative standard methodology for the recovery of enteric viruses from large volumes of tap water. The availability of efficient methods for detecting enteric viruses in drinking water will facilitate assessing the role of drinking water in the transmission of viruses.

James B. Lucas, Acting Director
Health Effects Research Laboratory

ABSTRACT

The tentative Standard Method (TSM) for concentrating enteric viruses from large volumes of drinking water and other finished waters was evaluated for nine enteric viruses using 100-gal (378-l) tap water volumes experimentally contaminated with about 100 plaque-forming units (PFU) of virus. The mean recoveries of poliovirus 1, coxsackieviruses A9 and B3, echovirus 7, bovine enterovirus 1, Minute Virus of Mice (parvovirus), reovirus 3, adenovirus 5 and simian adenovirus 11 were 22, 25, 2.5, 6.7, 22, 5.0, <1, <1 and <1%, respectively when the viruses were evaluated individually in succession. Although the recovery differences for the five enteroviruses suggested variable TSM effectiveness depending upon virus type, subsequent TSM experiments in which these viruses were simultaneously tested showed no significant recovery differences, averaging 16% (+2.7, 95% CI) overall. TSM recoveries for purified, dispersed mixtures of enteroviruses obtained from raw sewage and seeded into 100-gal volumes of tap water averaged only 3.9% which was approximately the same as poliovirus recoveries during the same time period.

Enterovirus recoveries showed marked seasonal variations that may be related to water quality changes. Reduced virus recovery was partly due to interference with virus adsorption to filters caused by soluble or colloidal tap water constituents that were removable by granular activated carbon. The effects of these interferences could be overcome by adding aluminum or magnesium chloride to the tap water to enhance virus adsorption.

Low virus recoveries by the TSM were also due to virus inactivation by the pH 11.5 glycine-NaOH used for eluting adsorbed viruses from filters. In this eluent the T-90 values (time for 90% virus inactivation) for Minute Virus of Mice, poliovirus, reovirus and the simian adenovirus SV-11 were 20, 1.8, <1 and <1 minutes, respectively.

Virus recoveries with TSM and other negatively charged filters could be markedly improved by various procedural modifications: (1) the use of aluminum or magnesium chloride to enhance virus adsorption, (2) the use of less alkaline glycine or beef extract both with and without EDTA for elution of viruses from primary adsorbents, and (3) recirculation of the eluent through the adsorbent filters. With the exception of MVM, viruses in beef extract eluents could be efficiently reconcentrated by organic flocculation.

Improved virus recoveries were also obtained with adsorbent filters that are more electropositive than TSM filters. Zeta Plus filters that are positively charged up to about pH 5 to 6 adsorbed >99% of various viruses from tap water at ambient pH levels of 7.0-7.5 without added polyvalent cation salts. Most viruses could be effectively eluted with glycine-NaOH, pH >9.5 or beef extract. Low levels of poliovirus in 100-gal (378-l) volumes of tap water were more effectively recovered with Zeta Plus filters than with the TSM using Cox filters.

SECTION 4

MATERIALS AND METHODS

VIRUSES, CELL CULTURES AND VIRUS ASSAYS

The nine laboratory viruses used in this study were poliovirus type 1, strain LSc (P-1), coxsackievirus types A9 (CA-9) and B3 (CB-3), echovirus type 7 (E-7), bovine enterovirus type 1 (BE-1), reovirus type 3 (R-3), adenovirus type 5 (Ad-5), simian adenovirus SV-11 (SV-11) and Minute Virus of Mice (MVM). Laboratory viruses were grown and assayed in the following cell cultures: P-1, CB-3 and E-7 in Buffalo Green Monkey Kidney (BGMK) cells; CA-9 in RD cells (American Type Culture Collection number 136); R-3, Ad-5 and SV-11 in VERO cells; BE-1 in secondary or tertiary bovine embryonic kidney cells; and MVM in the A9 strain of L cells. In addition, naturally occurring virus mixtures concentrated and purified from raw sewage were also used; they were assayed in BGMK cells. All cell cultures were grown and maintained in modified Eagle Minimum Essential Medium (MEM) with previously described supplements (38), except RD cells which were grown in MEM additionally supplemented with Eagle non-essential amino acids.

Except for MVM, monodispersed, purified stocks of laboratory viruses were prepared as previously described (17) and diluted in 0.01 M phosphate-buffered saline (PBS) for storage. MVM stocks were prepared by infecting non-confluent (6×10^4 cells/cm² of culture vessel surface area) cultures of A9 L cells at low multiplicity in order to obtain adequate infective virus yields. When A9-L cell monolayers exhibited 75-100% cytopathology, cultures were frozen and thawed three times to maximally disrupt cell membranes. Cell debris was removed by centrifugation at $10,000 \times g$ for 30 min, and extracted twice with TE buffer (50 mM tris(hydroxymethyl)aminomethane hydrochloride, 1.0 mM disodium ethylenediamine tetraacetic acid, pH 8.7). Combined TE buffer extracts were then extracted with Freon, as previously described (16). Freon-extracted MVM preparations were not subjected to sucrose density gradient centrifugation because electron microscopy revealed that they were monodispersed by Freon-extraction.

Virus stock suspensions were diluted in 0.01 M phosphate buffered saline to approximately 100 plaque-forming units (PFU)/ml and stored at -70°C until used. Stock titers were verified for each experiment by plaque assay of 1-ml volumes of stock in 3-5 replicate 75 cm² cell culture flasks as described below.

Purified, dispersed preparations of naturally occurring enteric virus mixtures were obtained by centrifuging 1-liter volumes of composite raw sewage at $5,000 \times g$ and 4°C for 1 hr, decanting the supernatant, resuspending the

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SECTION 1

INTRODUCTION

BACKGROUND

The potential transmission of human enteric viruses via drinking water and other waters is of considerable public health concern (1, 2, 3, 18, 36, 39). The viruses of major concern in water are those that infect the alimentary tract of man and in some cases animals, and are excreted in large quantities with the feces by infected persons. These are the so-called enteric viruses, and they include members of the following taxonomic groups: (1) enteroviruses (polio-, coxsackie- A and B and echoviruses), (2) reo- and rotaviruses, and (3) adenoviruses as well as the Norwalk-type agents and Hepatitis type A, which are not yet taxonomized due to limited information on morphology and chemical composition.

The continued occurrence of waterborne outbreaks of Hepatitis A and perhaps other virus diseases such as gastroenteritis (11, 43) as well as the apparent isolation of enteric viruses from drinking water supplies in the U.S. (22, 27) and elsewhere (10, 13, 30), indicate the need for a more thorough and systematic evaluation of the potential for virus disease transmission via drinking water. Evaluation of this problem requires the development and utilization of simple and reliable methods to quantitatively detect relatively small amounts of enteric viruses in large volumes of raw and finished drinking water supplies (34, 35).

Research efforts on methods to detect enteric viruses in water have produced two-step microporous filter adsorption-elution methods that are sometimes capable of concentrating small numbers of enteroviruses from 100 or more gallons of drinking water (14, 21, 24, 34, 36), and a tentative method of this type was introduced into the 14th edition of Standard Methods (1).

In this method the water is pressure filtered through microporous (0.45 μ to 8.0 μ pore size) filters having large surface areas to which viruses adsorb by a reversible reaction. The adsorption reaction is thought to be primarily electrostatic in nature, although weak attractive forces, such as hydrogen bonding, dipole-dipole interactions and hydrophobic effects may also be involved (26, 28). In order to enhance virus adsorption to the filter surfaces, appropriate ionic conditions are created in the flowing water by the controlled addition of acid and sometimes polyvalent cation salts prior to passage of the water through the adsorbent filter. After processing the desired volume of water, the adsorbed viruses that have accumulated on the filter surfaces are eluted with approximately one liter of an alkaline buffer solution consisting of pH 11.5 glycine-NaOH. The eluate obtained is readjusted

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by the addition of acid and AlCl_3 to ionic conditions that enhance virus adsorption: pH 3.5 and 0.0005 M AlCl_3 . The viruses are then adsorbed to a second, smaller (47-mm diameter) microporous filter series composed of fiberglass, asbestos and epoxy. The adsorbed viruses are then eluted from this filter with several milliliters of alkaline buffer solution. The virus-containing eluate is adjusted to a physiological pH and isotonicity and then assayed for viruses in cell cultures using conventional virological techniques.

The development of flow-through filter adsorption-elution systems for concentrating small quantities of viruses from large volumes of water represents a much needed technical achievement. However, many of the key variables involved in this type of methodology have not been adequately evaluated, and some of the procedures and equipment in current use have been somewhat arbitrarily selected. A rational and systematic evaluation of the methodology and materials is needed.

In particular, it has not been demonstrated that the tentative Standard Method (TSM) is capable of efficiently detecting viruses of all major enteric virus groups including Hepatitis A virus. The method was developed and evaluated primarily for enteroviruses, especially poliovirus, and there is information in the literature indicating that the method may be incapable of recovering some other enteric viruses. For example, in the application of filter adsorption-elution methods to adenoviruses, Fields (20) found that adenovirus type 5 was inactivated by the alkaline glycine-NaOH used for elution of adsorbed viruses.

Another deficiency concerning evaluation of the TSM is that the method has not been evaluated over a sufficient time period with the same viruses and water source to determine if changes in water quality influence its effectiveness. Dissolved and colloidal substances in water may interfere with virus recovery, and the types and concentrations of such interfering substances may vary over time (15, 16, 36, 40).

OBJECTIVES

General Objective

To rigorously and systematically evaluate the tentative Standard Method for concentrating and detecting enteric viruses in large volumes of drinking water and other finished waters.

Specific Objectives

To evaluate the tentative Standard Method for detecting and concentrating enteric viruses from tap water using the four different types of microporous filter systems that are currently recommended as virus adsorbents and representative viruses from the major enteric virus groups. The four primary virus adsorbent systems being evaluated are: (a) Balston fiberglass-epoxy filter tubes, (b) Cox fiberglass-asbestos-epoxy filter discs, (c) Millipore cellulose nitrate membranes, and (d) Commercial Filters fiberglass cartridge filters and Cox fiberglass-asbestos-epoxy filter discs in series. A total of nine different viruses are being tested: poliovirus, coxsackieviruses A9 and

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and B3, echovirus 7, bovine enterovirus 1, reovirus 3, simian adenovirus SV-11, (human) adenovirus 5 and Minute Virus of Mice (an enteric parvovirus).

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After evaluating the tentative Standard Method with the nine different laboratory strains of enteric viruses, the method will then be evaluated using mixtures of naturally occurring enteric viruses obtained from sewage.

If the present tentative Standard Method is found to be incapable of efficiently recovering certain enteric viruses, then modifications of the presently recommended methodology will be evaluated for their ability to efficiently detect representatives of all enteric virus groups.

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SECTION 2

CONCLUSIONS

1. The current tentative Standard Method (TSM) for enteric virus concentration from drinking water and other finished waters has serious deficiencies causing it to give poor virus recoveries.
2. One major deficiency of the TSM is that the pH 11.5 glycine-NaOH used to elute adsorbed viruses from filters rapidly inactivates adenoviruses and reoviruses. It also inactivates enteroviruses and parvoviruses, but not as rapidly. Alternative eluents which employ less alkaline (pH 9.5-10.5) conditions in glycine, proteinaceous media such as 0.3-3% beef extract, chelating agents such as EDTA, or combinations of these conditions show considerable promise for all major enteric virus groups. Virus elution efficiency is further improved when these alternative eluents are recirculated through the adsorbents, thereby increasing the contact time.
3. Another serious deficiency of the TSM causing reduced virus recoveries is that soluble and/or colloidal substances in finished tap water can interfere with virus adsorption to conventional, electronegative TSM primary virus adsorbents. There appear to be temporal changes in magnitude of these interfering effects that may be related to seasonal changes in the nature and amount of interfering substances in tap water. The effects of these interfering substances can be overcome by adding polyvalent cation salts, such as $MgCl_2$ and $AlCl_3$, to the tap water to enhance virus adsorption to TSM primary virus adsorbents and the Filterite pleated cartridge fiberglass-epoxy filter.
4. The four alternative primary virus adsorbents of the TSM do not differ significantly in terms of virus recovery efficiency, although recoveries with the Balston filter were frequently lower than those with the other three adsorbent systems. The primary virus adsorbent systems did differ in terms of their susceptibility to clogging, with the Millipore and Carborundum-Cox systems being more susceptible than the Balston and Cox systems.
5. The results of this study suggest that all enteroviruses are apparently recoverable with equal effectiveness by the TSM. This conclusion is based on finding: (1) that the recovery efficiencies for five different laboratory-grown enteroviruses did not differ significantly in simultaneous experiments and (2) that recovery efficiencies for natural enterovirus mixtures obtained from sewage were not significantly different from recovery efficiencies for exogenously added, laboratory-grown poliovirus during the same time period.

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6. Microporous filters that are more electropositive than the current TSM filters and are composed of diatomaceous earth-cellulose-"charge-modified" resin mixtures appear to offer distinct advantages as virus adsorbents because: (1) they efficiently adsorb viruses from tap water at neutral pH without added polyvalent cation salts, (2) they are less affected by soluble and/or colloidal interferences in tap water that reduce virus adsorption, and (3) with the possible exception of adenoviruses, adsorbed viruses can be readily eluted with either pH 9.5 to 10.5 glycine-NaOH or 0.1 to 3% beef extract, pH 9 to 10.
7. Highly electropositive microporous filters composed of asbestos-cellulose mixtures may also be effective virus adsorbents because they efficiently adsorb viruses over a wide pH range without added salts and the adsorbed viruses can be eluted with pH 9 to 10 beef extract.

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SECTION 3

RECOMMENDATIONS

1. Because the current TSM for concentrating enteric viruses from drinking water and other finished waters is relatively ineffective, improved filter adsorption-elution methods that will be suitable and effective in field use should be developed and evaluated.
2. Further efforts should be made to develop and evaluate alternative eluents to pH 11.5 glycine-NaOH. In particular, the use of glycine-NaOH at lower pH levels and 0.1 to 3% beef extract with and without chelating agents such as EDTA or other additives should be investigated.
3. Further studies should be done with beef extract and other proteinaceous eluents to improve the organic flocculation procedure for parvoviruses.
4. Because polyvalent cation salts such as $MgCl_2$ and $AlCl_3$ are capable of overcoming the effects of the soluble and/or colloidal interferences in tap water that reduce virus adsorption efficiency, it may be prudent to routinely use salt enhancement of virus adsorption, unless it can be experimentally demonstrated the efficient virus recoveries from large volumes of a particular water are obtained without added salts.
5. Electronegative fiberglass-epoxy pleated cartridge filters (Filterite) should be considered as a fifth alternative primary virus adsorbent because: (1) they have a large surface area and, hence, a large filtration-volume capacity for water; (2) they efficiently adsorb viruses under the same conditions as TSM filters, and (3) the adsorbed viruses are elutable with pH 10.5 glycine-NaOH and beef extract.
6. Additional studies should be done to further develop and evaluate improved and simplified filter adsorption-elution methods for virus concentration from water based on the use of electropositive adsorbent filters.
7. The physico-chemical mechanisms of virus adsorption to and elution from filters and other surfaces should be investigated in order to provide a rational basis for the further development and optimization of filter adsorption-elution methods to concentrate enteric viruses from water. In addition, the chemical characteristics and behavior of the dissolved and/or colloidal substances in tap water that interfere with virus adsorption to filters should also be fully investigated in order to develop optimum methods for their elimination or control.

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sediment in 20 ml of PBS and extracting for 2 min with $\frac{1}{2}$ volume of Freon. The mixture was centrifuged at $2,000 \times g$ and 4°C for 15 min to break the emulsion and the aqueous supernatant was combined with the first supernatant. This sample was centrifuged at $4,000 \times g$ and 4°C for 1 hr. to remove large particles including large virus aggregates, and the resulting supernatant was collected, treated with antibiotics (400, 170 and $6.3 \mu\text{g/ml}$ of Kanamycin, Gentamycin and Fungizone, respectively) and saved as purified, dispersed natural virus stock. All dispersed preparations of laboratory and field viruses were stored at 4 or -70°C until used. Virus titers were verified for each experiment by 3-5 replicate inoculations of 3-ml volumes in 75 cm^2 cell culture flasks.

All enteroviruses and natural virus mixtures were assayed by the procedures previously described for poliovirus and R-3 and SV-11 were assayed by procedures previously described for these viruses (38). For assays of Ad-5, confluent monolayers of VERO cells were pretreated for 24 hr with 0.05 mg/ml 5-iodo-2'-deoxyuridine (IDU) in MEM. After virus inoculation and a 1-2 hr adsorption period, assays were completed by the double overlay procedure previously described for SV-11 (38). MVM was assayed by inoculating drained, 2-6 hr old A9 L cell cultures containing 6×10^4 cells/ cm^2 of flask surface area. After 1-2 hr at 37°C for virus adsorption, the cultures were overlaid with a medium consisting of MEM, 7.5% heat-inactivated fetal calf serum, 1.0% agar, 0.4% NaHCO_3 , 0.017% pancreatin and 2X antibiotics. After incubating for 5-7 days at 37°C , a second overlay containing 0.0025% neutral red was applied. The cultures were reincubated at 37°C and plaques were counted over the following several days.

Samples containing relatively low virus levels were assayed by inoculating 1- or 3-ml volumes into 25 or 75 cm^2 cell culture flasks, respectively, and then allowing 1-2 hr for virus adsorption. After adsorption the cultures were inoculated with agar overlay media and incubated as described in the previous paragraph. Plaques were counted over a period of several days until no further increase in PFU was observed.

VIRUS CONCENTRATION PROCEDURES

Single Step Experiments

In virus concentration experiments involving tap water volumes of 12 liters or less, 47-mm diameter virus adsorbent filters were used in a single-step concentration procedure. The following adsorbent filters were used: (1) fiberglass-asbestos-epoxy (Series AA, Type M-780, Cox Instrument Div., Lynch Corp., Detroit, Mich.); (2) fiberglass-epoxy (No. 8025-035, Filterite Corp., Timonium, Md.); (3) cellulose-diatomaceous earth-"charge modified" resin (Series S, Zeta Plus, AMF Cuno, Meriden, Ct.); and (4) asbestos-cellulose (Seitz, Grade S, Alsop Engineering Corp., Milldale, Ct.). Filter pore sizes ranged from 8.0 to $0.2 \mu\text{m}$. In these experiments, volumes of cold Chapel Hill tap water were dechlorinated with 10 mg of sodium thiosulfate per liter. Portions were adjusted to desired pH levels with 1.0 or 0.1 N HCl or NaOH, and in some experiments small volumes of concentrated stock solutions of MgCl_2 or AlCl_3 were added to desired final concentrations.

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Samples were seeded with a small volume of monodispersed stock virus to

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give a desired virus input. Depending upon their volumes, the samples were placed in either 600-ml capacity polycarbonate or 20-liter capacity stainless steel pressure vessels, and if the input virus levels were high, small samples were taken for virus assay. The samples were filtered through microporous filters at the desired flow rate using a regulated nitrogen gas cylinder as a source of positive pressure. When the virus inputs were high, the filtrates were collected and assayed for viruses. Adsorbed viruses were recovered from the filters by filtering two successive 7.5-ml volumes of eluent fluid through the filters and collecting the eluates dropwise. The pH of the combined eluate was measured and adjusted to pH 7.4 with 0.05 M glycine-HCl, pH 1.5. After measuring the total volume, the eluate was assayed for viruses.

Two Step TSM Experiments

Viruses were concentrated from 100-gallon (378-liter) volumes of experimentally contaminated Chapel Hill tap water by the two-stage procedures described in Standard Methods (1, 21, 24). In these experiments, monodispersed viruses were continuously added at approximately 1 PFU/gal to the flowing tap water along with the sodium thiosulfate solution used for dechlorination via one pump of a triplex fluid proportioner (Johanson and Son Machine Corp., Clifton, N.J.). To acidify the tap water prior to virus adsorption, HCl or mixtures of HCl plus $AlCl_3$ or $MgCl_2$ were added to the flowing tap water using two alternating pumps of the fluid proportioner. In some experiments, the four alternative, primary virus adsorbent systems of the TSM were simultaneously tested with the same tap water and viruses at flow rates of about 2.5 gallons per minute. The TSM primary virus adsorbent systems were (1) "Millipore": 293-mm diameter, 8- and 1.2- μ m porosity mixed cellulose ester filter series (Millipore Corp., Bedford, Mass.); (2) "Balston": 1-inch diameter x 7-inch long, 8- μ m porosity, fiberglass-epoxy tube filter (Grade C, Balston, Inc., Lexington, Mass.); (3) "Cox": 267-mm diameter, 5- and 1- μ m porosity, fiberglass-asbestos-epoxy filter series (Type M-780, series AA, Cox Instrument Div., Lynch Corp., Detroit, Mich.); and (4) "Carborundum Cox": 2.5-inch diameter x 10-inch long, 3- μ m porosity, fiberglass yarn, honeycomb cartridge filter (Model K27R10S, Commercial Filters Division, Carborundum Co., Lebanon, Indiana) followed by a 127-mm diameter Cox filters series as specified in number 3 above.

In other two-stage TSM experiments, four identical primary virus adsorbent systems (specifically, 267-mm diameter 5- and 1- μ m porosity Cox filters) were used to simultaneously test four or five different viruses under the same water quality conditions. When five viruses were simultaneously tested with four replicates of a single type of adsorbent, two different viruses having different host cell requirements for plaque assay were mixed together in the sodium thiosulfate feed solution for one adsorbent. After processing, portions of the final concentrate were separately assayed for each virus.

Viruses were eluted from the primary adsorbents with one liter of eluent fluid, and the collected eluates were adjusted to approximately neutral pH with 0.05 M glycine-HCl, pH 1.5. Viruses in these primary eluates were reconcentrated either by microporous filter adsorption-elution according to the TSM (1) using specific procedures similar to those for the single-stage concentration procedure described above or by an alternative procedure such as "organic

flocculation" (25).

Virus input levels were calculated for each experiment from virus stock titer, volume of stock virus added to the thiosulfate dosing solution and volume of thiosulfate solution used.

Two Step Experiments with Modified Procedures

In still other two-stage filter adsorption-elution experiments, various primary virus adsorbents were simultaneously evaluated with one or more viruses. In addition to the TSM primary virus adsorbents and 293-mm diameter Zeta Plus filters, a 2.5-inch diameter x 10-inch long, 0.45- μ m porosity, melamine impregnated paper-fiberglass-epoxy pleated cartridge filter (Duo-Fine, Filter-ite Corp., Timonium, Md.) was also tested. Zeta Plus filters were sometimes used with a 2.5-inch diameter x 10-inch long, 1- μ m porosity polypropylene yarn honeycomb cartridge pre-filter (Model 039R10S, Commercial Filters Division). Viruses were eluted from the filters with 0.2 to 1.0 liters of eluent fluid consisting of either 0.05 M glycine-NaOH, pH 9-11.5 or 0.3-3% beef extract or nutrient broth, pH 7-10.5. In some cases, the eluent fluid also contained ethylene diamine tetraacetic acid (EDTA), 0.005 to 0.1 M. Eluent fluids were either slowly passed once through the virus adsorbent as specified in the TSM or they were slowly recirculated for a specified time period either manually or with the aid of a peristaltic pump.

ELECTROPHORESIS OF FILTER MEDIA

The electrophoretic mobility of filter media as a function of pH was determined by Briggs cell microelectrophoresis using the procedure of Black and Smith (5). Filter media were blended in distilled water for 1 to 5 minutes and either filtered through gauze or allowed to settle in order to obtain particle suspensions of suitable size for electrophoretic analysis. Suspensions were buffered with 0.001 M acetate at pH 5, phosphate at pH 7, and borate at pH 9. Sodium chloride was added to a final 0.05 M concentration to assure constant ionic strength.

STATISTICAL ANALYSES

Statistical analyses were performed according to procedures described by Dixon and Massey (12) using a Texas Instrument (Dallas, Texas) SR-52 programmable calculator and statistics library ST-1 (42). Differences were considered significant only when $P \leq 0.05$ for the appropriate test.

WATER

Finished Chapel Hill tap water was obtained from a four place manifold connected to a 1-inch diameter water line in the laboratory. Each position on the manifold served a separate fluid proportioner. The raw water source for Chapel Hill tap water is University Lake, a 200-acre impoundment on Morgan Creek that was constructed in 1932. When full, University Lake has a maximum depth of 30 feet and a mean depth of 9.25 feet. As is typical for many small southeastern lakes located in highly vegetated river basins, University Lake is nutrient-rich and contains considerable humic material. The lake is

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monomictic with a thermocline and clinograde developing during the summer. Stratification persists into the late fall with turnover usually occurring in November. Circulation and mixing continue through the winter and spring producing high levels of dissolved oxygen (DO). During the summer, reducing conditions and low DO levels prevail below the thermocline in the hypolimnion with high levels of iron, manganese and humic substances. During the summer months of 1976 and 1977, severe drought conditions caused the lake level to drop precipitously, resulting in degradation of raw water quality due to the use of raw water from below the thermocline. During this period, supplemental finished drinking water was obtained from the city of Durham which is also served by a small surface impoundment on the Flat River called Lake Michie. The Chapel Hill water treatment plant has a multilevel intake structure that allows raw lake water to be drawn from the depths having the best water quality. During the summer, water is usually drawn from the euphotic zone, thereby avoiding the poorer quality, reduced water in the hypolimnion. In both Chapel Hill and Durham, the raw water is treated by a conventional scheme consisting of alum coagulation-flocculation, sedimentation, mixed media (anthracite-sand) filtration and chlorination. In the warmer summer months, potassium permanganate is sometimes added at the coagulation step for taste and odor control. Storage time of the finished water at the Chapel Hill and Durham plants is generally about 1 and 6 hours, respectively. In the Chapel Hill distribution system, water towers may provide as much as 1 day of storage, depending upon demand. Typical water quality data for raw and finished Chapel Hill water are given in Table 1. Neither lake nor its' watershed receives appreciable amounts of domestic or industrial effluents.

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TABLE 1. QUALITY OF RAW AND FINISHED CHAPEL HILL DRINKING WATER

Year	Months	Turbidity (NTU)		Color (Cobalt Units)		pH	Total Alkalinity (mg/l as CaCO ₃)		Total Hardness (mg/l as CaCO ₃)		Total Iron (mg/l)		Total Manganese (mg/l)		Total Phosphate (mg/l)		Total Coliforms (CFU/100ml)		
		Raw	Fin.	Raw	Fin.		Raw	Fin.	Raw	Fin.	Raw	Fin.	Raw	Fin.	Raw	Fin.	Raw	Fin.	
1976	6-7	12.9	0.13	23.6	0.02	7.4	7.1	25.2	21.5	29.4	35.2	0.22	0.02	0.10	TC	0.92	1.76	15.5	0
	8-9	12.4	0.08	24.2	0	7.1	7.1	28.2	28.2	31.6	33.4	0.28	0.02	0.12	0.05	0.11	1.44	10.0	0
	10-11	18.7	0.07	40.7	1.40	7.2	7.0	35.7	34.7	31.6	32.2	0.43	0.07	0.30	0.04	ND	1.65	9.0	0
	12-1	17.8	0.10	42.0	0	7.0	7.2	26.0	28.5	32.0	32.0	0.35	0.04	0.18	0.02	1.14	ND	12.4	0
1977	2-3	26.4	0.12	55.0	0	7.0	7.2	19.0	19.0	26.0	26.5	0.42	0.06	0.15	0.02	ND	1.71	23.6	0
	4-5	13.7	0.09	35.7	0	7.0	7.1	21.2	22.0	22.4	22.4	0.36	0.04	0.19	0.02	ND	1.86	19.8	0
	6-7	10.6	0.08	39.0	0	7.1	7.1	29.5	28.5	25.0	26.5	0.74	0.02	0.26	0.01	ND	0.63	14.6	0
	8-9	18.1	0.11	86.5	0	6.9	7.2	28.5	30.5	26.5	28.0	0.26	0.04	0.24	0.03	ND	1.68	38.6	0
1978	10-11	18.4	0.12	136.0	0.04	7.0	7.3	27.0	30.5	27.0	26.5	0.55	0.04	0.33	0.04	1.80	ND	40.3	0
	12-1	28.9	0.22	185.5	0.20	6.8	7.3	20.5	24.0	27.0	27.0	0.50	0.04	0.02	0.01	ND	1.34	ND	0
	2-3	45.5	0.24	185.5	0.20	6.4	7.2	15.0	19.0	23.5	24.0	0.66	0.05	T	0	0.65	ND	25.5	0
	4-5	32.0	0.16	24.5	0.06	6.4	7.2	16.5	17.0	21.0	21.0	0.62	0.04	T	0	0.53	0.49	49.5	0
1979	6-7	16.5	0.11	36.0	0.06	6.6	7.2	23.0	30.5	26.5	26.5	0.51	0.04	0.08	T	0.47	0.49	18.0	0
	8-9	21.5	0.26	18.5	0.30	6.7	7.5	28.5	36.0	24.5	24.0	0.35	0.04	0.15	T	ND	0.55	27.0	0
	10-11	23.0	0.16	12.5	0.42	6.6	7.5	32.0	39.5	29.5	29.0	0.58	0.05	0.49	T	ND	0.73	11.0	0
	12-1	22.0	0.28	16.0	1.05	6.8	7.5	31.0	36.0	31.0	32.0	0.42	0.05	0.05	T	ND	ND	5.2	0
Mean	1976	20.1	0.14	55.8	0.24	6.9	7.2	25.4	27.8	27.2	27.9	0.45	0.04	0.17	0.02	0.80	1.19	23.0	0
	1977	18.7	0.07	12.5	0	6.4	7.0	15.0	17.0	21.0	21.0	0.22	0.02	T	0	0.11	0.49	5.2	0
S.D.	1976	45.5	0.28	185.5	1.40	7.4	7.5	35.7	39.5	32.0	35.2	0.74	0.07	0.49	0.05	1.80	1.86	49.5	0

S. Coliforms assayed by MF method; colony-forming units

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RESULTS AND DISCUSSION

TSM EVALUATION WITH 100-GALLON TAP WATER VOLUMES AND INDIVIDUAL LABORATORY VIRUSES

A series of consecutive experiments was conducted in which the two-stage TSM was evaluated for the recovery of relatively low levels of individual laboratory-grown enteric viruses using 100-gallon tap water volumes and the simultaneous parallel operation of the four, alternative primary virus adsorbent systems. As planned in the original project design, a series of consecutive TSM experiments was usually completed for a single laboratory virus before initiating a series of experiments with another laboratory virus.

The mean virus recovery efficiencies for the consecutive evaluation of the five different enteroviruses over a 15-month period are given in Table 2 for each of the four primary virus adsorbent systems along with the average results for all four systems. Detailed data of the individual experiments are given in Tables A1 through A5 of the Appendix. The overall mean virus recovery for all five viruses and all four primary virus adsorbent systems was 16% (+ 2.7, 95% confidence interval). For P-1, C-B3, C-A9 and EE-1, analysis of variance gave no significant difference in virus recovery efficiency among the four alternative primary virus adsorbents ($P > 0.2$ in all cases). However, for E-7 the differences in virus recovery efficiency among the four primary virus adsorbents were significant ($P = 0.01$). Examination of the results of the individual E-7 experiments suggested that virus recovery efficiency might be related to filter clogging as indicated by the volume of tap water processed. Virus recoveries were generally higher for the primary virus adsorbents that clogged (Millipore and Carborundum/Cox) than for those that did not clog (Balston and Cox).

As further evidence for this effect, the E-7 runs were divided into two groups: those where all four primary adsorbents processed 100 gallons and those where one or more primary adsorbents processed less than 100 gallons due to premature clogging. Within each group, the differences in E-7 recovery efficiency among the four primary adsorbents were tested for significance by analysis of variance. In runs where less than 100 gallons was processed by one or more primary adsorbent systems, the differences in E-7 recovery efficiencies among the four primary adsorbents were significant ($P = 0.01$), while in runs where all primary adsorbents processed 100 gallons, the differences in E-7 recovery efficiencies were not significant ($P = 0.56$). Furthermore, when the results of the runs where one or more primary adsorbents processed less than 100 gallons were grouped into two sets: Balston

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plus Cox (100 gallons always processed) and Millipore plus Carborundum/Cox (< 100 gallons processed by one or both) the differences in E-7 recovery efficiencies were significant at the 5% level in a paired t -test ($P = 0.001$). The reasons for higher E-7 recoveries with clogged filters are unclear and deserve further study. It is possible that more adsorption sites are still available on filters processing less than 100 gallons because less tap water interferences accumulate due to the lower volume processed. Alternatively, accumulated filter clogging materials may themselves be responsible for improved virus adsorption, either by providing additional virus adsorption sites or by reducing the porosity of the filter and thereby increasing virus retention.

The results in Table 2 indicate that there are appreciable differences in recovery efficiency between the five different enteroviruses, regardless of the type of primary virus adsorbent system. Analysis of variance for each filter type over the five enterovirus types ($v_1 = 4$, $v_2 = 41$) confirmed the differences (Balston: $F = 4.797$, $P = 0.003$; Cox: $F = 3.292$, $P = 0.020$; Carborundum-Cox: $F = 3.327$, $P = 0.019$; Millipore: $F = 3.004$, $P = 0.029$). Considering that all five viruses belong to the same group, the substantial differences in virus recovery efficiency were unexpected, and they suggested the disturbing possibility that there might be wide variations in recovery efficiency for the more than 70 types of human enteroviruses. However, subsequent simultaneous testing of viruses (described in the next section) indicated no differences in recovery among these viruses.

The results of a series of consecutive TSM experiments conducted over a 9-month period with relatively low levels of the other four laboratory-grown enteric viruses are summarized in Table 3. Detailed data of the individual runs are given in Tables A6 through A9 of the Appendix. For each virus it is apparent that recovery efficiencies for the four alternative primary adsorbent systems do not differ and for MVM this was confirmed by analysis of variance ($P = 0.85$). More important, however, is the fact that the two adenoviruses and reovirus type 3 were not recovered at all, and the parvovirus, MVM, was recovered with an average efficiency of only 5.0%. The recoveries are also considerably lower than the overall mean enterovirus recovery of 16%. Thus, enteroviruses, including poliovirus, may not be suitable models for determining the ability of filter adsorption-elution methods to recover adeno-, reo- and parvoviruses. It should be noted, however, that because the individual viruses were consecutively tested, it is not possible to attribute the observed virus recovery differences entirely to differences in virus behavior under TSM conditions. Temporal changes in water quality and perhaps other conditions also may have contributed to the observed virus recovery differences.

TSM EVALUATION WITH SIMULTANEOUS TESTING OF LABORATORY VIRUSES

If the recovery efficiencies obtained in the consecutive evaluation of individual enteroviruses in 100-gallon, two-step TSM experiments during the first two years of the project are plotted as a function of time, there appear to be time-related changes in recovery efficiency (Figure 1). The bi-monthly average enterovirus recovery efficiencies range from 2.0 to 32%. Analysis of variance for mean percent virus recovery by all filter types over bi-monthly intervals confirmed the variations ($F = 13.195$; $v_1 = 6$, $v_2 = 177$; $P = 2.51 \times 10^{-12}$).

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TABLE 2. ENTEROVIRUS RECOVERIES FROM 100-GALLON VOLUMES OF EXPERIMENTALLY CONTAMINATED TAP WATER BY THE TENTATIVE STANDARD METHOD

Enterovirus	Experimental No. Runs	Balston			Cox			Carbonadium-Cox			Millipore			All Filters		
		Mean Vol. (gal)	Mean Rec. (%)	Mean Input (PFU)	Mean Vol. (gal)	Mean Rec. (%)	Mean Input (PFU)	Mean Vol. (gal)	Mean Rec. (%)	Mean Input (PFU)	Mean Vol. (gal)	Mean Rec. (%)	Mean Input (PFU)	Mean Vol. (gal)	Mean Rec. (%)	Mean Input (PFU)
Polio-virus type 1	6-24-76 to 9-23-76	13	15	(± 7.1)	101	70	20	98	68	29	100	69	25	100	69	22
							(± 11)			(± 17)			(± 16)			(± 6.3)
Coxsackie-virus type 83	10-13-76 to 11-03-76	7	2.6	(± 2.2)	100	56	3.7	100	56	2.8	100	56	0.7	100	56	0.7
							(± 1.6)						(± 1.2)			(± 1.1)
Echo-virus type 7	1-20-77 to 3-08-77	10	2.5	(± 2.5)	100	112	5.2	89	99	9.2	72	77	10	90	99	6.7
							(± 4.5)			(± 4.5)			(± 6.4)			(± 2.1)
Coxsackie-virus type A9	4-15-77 to 6-03-77	8	16	(± 5.8)	101	91	27	98	89	32	100	91	27	100	90	25
							(± 14)			(± 14)			(± 16)			(± 5.9)
Bovine enterovirus type 1	6-30-77 to 9-20-77	8	16	(± 10)	101	108	35	100	107	28	98	104	18	100	107	21
							(± 22)			(± 26)			(± 13)			(± 7.8)

^aAverage volume of tap water processed through adsorbent, in some experiments 100 gallons were not processed due to clogging of the filters (see Tables A1 through A5 in Appendix).

^bVirus inputs calculated from virus stock titers.

^cMean percent virus recovery: (95% confidence interval).

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TABLE 3. ENTERIC VIRUS RECOVERIES FROM 100-GALLON VOLUMES OF EXPERIMENTALLY CONTAMINATED TAP WATER BY THE TENTATIVE STANDARD METHOD

Enteric Virus	Experimental Period	No. Runs	Balston			Cox			Carborundum-Cox			Millipore			All Filters		
			Mean Vol. (gal)	Mean Input (PFU)	Mean Rec. (%)	Mean Vol. (gal)	Mean Input (PFU)	Mean Rec. (%)	Mean Vol. (gal)	Mean Input (PFU)	Mean Rec. (%)	Mean Vol. (gal)	Mean Input (PFU)	Mean Rec. (%)	Mean Vol. (gal)	Mean Input (PFU)	Mean Rec. (%)
Minute Virus of Mice	10-11-77 to 04-20-78	6	100	74	6.0 (±13)	102	74	1.7 (±3.1)	100	66	7 (±15)	95	81	5.1 (±11)	99	79	5.0 (±4.4)
Siebia Adeno-virus SV-11	11-16-77 to 01-16-78	6	98	115	0	100	120	0	97	118	0	97	119	0	98	118	0
Adeno-virus type 5	01-10-78 to 03-10-78	4	100	116	0	100	73	0	83	113	0	93	101	0	94	102	0
Reo-virus type 3	04-04-78 to 06-19-78	6	100	109	0	100	102	0	100	109	0	101	107	0	100	107	0

Average volume of tap water processed through adsorbent; in some experiments 100 gallons were not processed due to clogging of the filters (see Tables A6-A9 in Appendix).

Virus inputs calculated from virus stock titers.

Mean percent virus recovery; (95% confidence interval).

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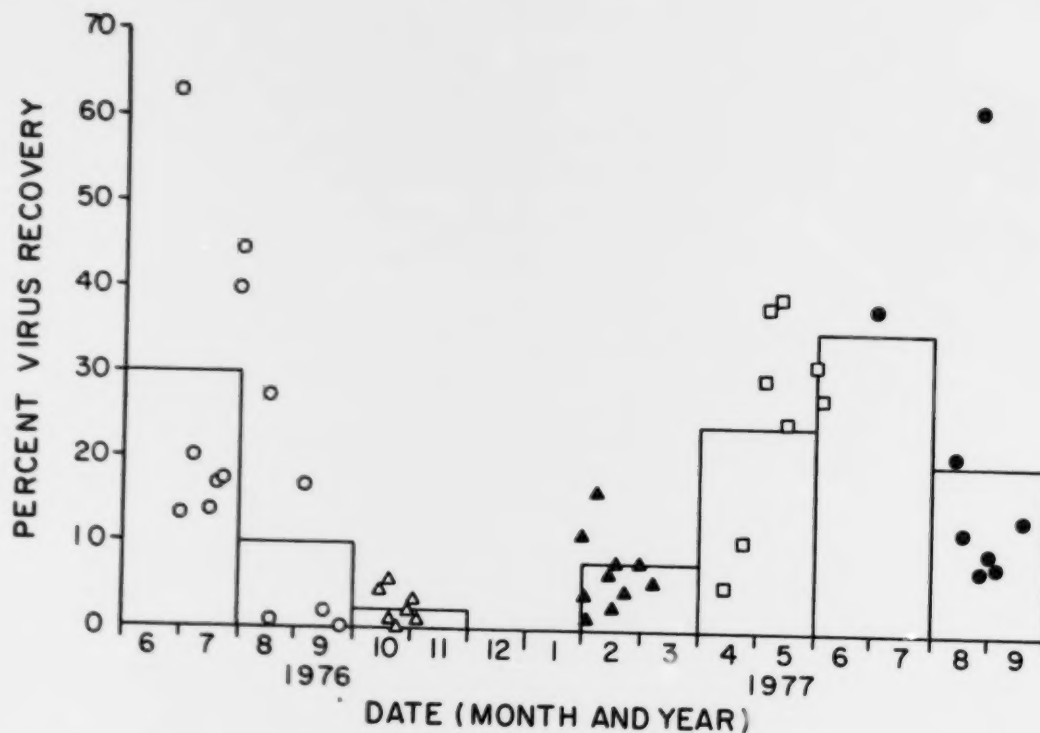


Figure 1. Enterovirus recoveries from 100-gallon volumes of experimentally contaminated tapwater, grouped into bimonthly periods. (June 1976-September 1977). Polio 1(○)/CB-3(△)/CA-9(□)/BE-1(●)/ECHO 7(▲)

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In order to determine if these observed differences in enterovirus recoveries were due to real differences in the viruses or to other factors, such as temporal changes in water quality, a series of experiments was done during the third and final year of the study in which the enteroviruses were simultaneously tested with a single type of adsorbent system, namely the Cox filter series. Two-stage, 100-gallon (378-liter) experiments were done using TSM adsorption and elution conditions. The results of the experiments with the five enteroviruses are summarized in Table 4, and further detailed in Table A10 of the Appendix. Although the results of the experiments show differences in mean recovery efficiencies for the 5 different enteroviruses (Table 4), these differences were not significant by analysis of variance ($P = 0.111$). Thus, the previously observed differences in enterovirus recovery efficiencies with the TSM when evaluating viruses individually were apparently due to factors other than actual virus-specific differences.

A series of simultaneous, two-stage, 100-gallon (378-liter) TSM experiments was also done during the third and final project year with the other four laboratory enteric viruses in order to determine if the previous results from the consecutive evaluation of these viruses could be confirmed. The primary virus adsorbent was again the Cox filter series and poliovirus was simultaneously evaluated as a fifth virus to act as a control. The experiments were analyzed in two separate groups: those in which the two adenoviruses, R-3 and P-1 were simultaneously tested and those in which MVM and P-1 were simultaneously tested. The results for both groups are summarized in Table 5 and further detailed in Tables A12 and A13 of the Appendix. As in the previous experiments in which the viruses were individually tested, SV-11 and Ad-5 were not recovered at all. R-3 was just barely detected with no viruses recovered in 5 of 6 experiments. P-1 was recovered with an average efficiency of 11%, which is not significantly different by two-sample t -test ($P = 0.337$) than the recovery efficiency in the previous simultaneous experiments (Table 4).

In the second group of experiments, MVM recovery efficiencies averaged only 3.1% while those for P-1 averaged 11% (Table 5). Although MVM recoveries averaged somewhat lower than those in the previous consecutive experiments (Table 3), the differences were not significant by two-sample t -test ($P = 0.127$). P-1 recoveries were somewhat higher than those in previous simultaneous experiments (Table 4) but again the differences were not significant by two-sample t -test ($P = 0.337$). Comparison of mean recovery efficiencies for P-1 and MVM by two-sample t -test showed no significant difference ($P = 0.253$). Thus, the apparent reduced efficiency of the TSM for recovering MVM, compared to the representative enteroviruses, cannot be attributed to virus-specific differences. However, the TSM is apparently totally ineffective for recovering adenoviruses and reoviruses due to virus-specific factors.

TSM EVALUATION WITH LARGE VOLUMES OF TAP WATER AND LOW LEVELS OF NATURAL ENTERIC VIRUS MIXTURES OBTAINED FROM RAW SEWAGE

The results of a series of 100-gallon TSM experiments conducted over a 12-month period with relatively low levels of natural virus mixtures obtained from raw sewage in which the four alternative primary virus adsorbent systems were simultaneously evaluated are given in Table 6. Virus recoveries were

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TABLE 4. ENTEROVIRUS RECOVERIES FROM LARGE VOLUMES OF TAP WATER BY THE TWO-STEP TSM WHEN TESTING VIRUSES SIMULTANEOUSLY^a

Virus Type	No. ^b Runs	Tap Water Vol. (gal) ^c	Mean Virus Input (PFU) ^d	Mean Virus Recovery (%) ^e
Poliovirus type 1	9	103	132	7.5 (±9.0)
Bovine entero- virus 1	14	106	167	9.1 (±5.4)
Coxsackie- virus B3	14	108	283	15 (±10)
Coxsackie- virus A9	4	99	127	33 (±30)
Echovirus type 7	14	108	192	23 (±18)

^aPrimary virus adsorbent: 267- or 293-mm dia., 5- and 1- μ m pore size Cox filter series.

^bNumber of runs varies because some data were not obtained due to occasional assay failure or stock problems resulting in inputs of <20 PFU.

^cMean tap water volume processed through adsorbent.

^dVirus input levels calculated from virus stock titers.

^eMean percent virus recovery (95% confidence interval). See Table A10 in Appendix.

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TABLE 5. ENTERIC VIRUS RECOVERIES FROM LARGE VOLUMES OF TAP WATER BY THE TWO-STEP TSM WHEN TESTING VIRUSES SIMULTANEOUSLY^a

Virus Type	No. Runs ^c	Mean Tap Water Vol. (gal)	Mean Virus Input (PFU)	Mean Virus Recovery (%) ^b
Poliovirus type 1	10	108	77	11 (± 14)
Reovirus type 3	6	111	326	0.1 (± 0.2)
Adenovirus type 5	11	105	201	0
Simian Adeno- virus SV-11	10	104	282	0
Poliovirus type 1	9	105	126	11 (± 16)
Minute Virus of Mice	11	104	372	3.1 (± 4.0)

^aPrimary virus adsorbent: 267- or 293-mm dia., 5- and 1- μ m pore size, Cox filter series; virus input was calculated from virus stock titration.

^bMean percent virus recovery (95% confidence interval). See Tables A11 and A12 in Appendix.

^cNumber of runs varies among viruses because some data were not obtained due to occasional assay failure or virus stock problems resulting in inputs of less than 20 PFU/100 gal.

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TABLE 6. RECOVERY OF NATURAL ENTERIC VIRUS MIXTURES FROM 100-GALLON VOLUMES OF EXPERIMENTALLY CONTAMINATED TAP WATER BY THE TSM

Date	Run No.	Millipore		Balston		Cox		Carborundum-Cox		All Filters ^a	
		Virus Input (PFU)	Virus Rec. (%)	Virus Input (PFU)	Virus Rec. (%)	Virus Input (PFU)	Virus Rec. (%)	Virus Input (PFU)	Virus Rec. (%)	Virus Input (PFU)	Virus Rec. (%)
12-19-77	1	50	<2.2	42	<2.7	32	3.6	41	<2.7	41	0.9
01-30-78	2	22	5.2	21	<5.4	20	<5.5	9.5	11.8	18	4.3
02-01-78	3	14	8.1	25	<4.6	25	<4.5	5.9	<19	17	2.0
02-15-78	4	36	3.1	40	2.8	41	<2.7	14	<8.1	33	1.5
04-26-78	5	15	7.3	22	15.5	21	<5.2	22	4.8	20	6.9
04-28-78	6	23	9.8	33	3.7	32	<3.2	32	<3.2	30	3.4
06-13-78	7	40	<3.4	42	2.8	41	26	38	<2.8	40	7.3
06-27-78	8	16	<6.3	18	5.9	19	6.7	18	<5.8	18	3.2
07-14-78	9	13	18	14	<8.3	13	8.1	12	9.0	13	8.8
07-26-78	10	13	<7.9	14	<8.1	12	<9.2	13	<8.7	13	0
08-07-78	11	70	1.6	68	3.3	64	7.4	62	7.4	66	4.9
08-21-78	12	39	<3.3	41	<3.1	39	5.8	37	9.5	39	3.8
09-07-78	13	33	<3.3	36	<3.1	38	<2.9	31	<3.5	35	0
09-29-78	14	87	1.2	92	6.2	86	1.5	83	8.1	87	4.3
10-09-78	15	42	6.0	42	2.6	41	5.3	40	14	42	6.4
10-23-78	16	143	<0.7	145	<0.7	142	0.9	129	1.0	140	0.5
11-10-78	17	38	12	37	<2.9	40	2.7	35	16	37	7.8
	Mean ^a	41	4.3	43	2.4	42	4.0	37	4.8	40	3.9

^aLess-than values assigned values of 0% for calculation of means.

generally low, ranging from <1 to 26% and averaging only 3.9%. In similar experiments with laboratory grown poliovirus type 1 done during the same time period but on different days (see Table A13 of the Appendix), recovery efficiencies ranged from <1 to 47% and averaged 4.1%. No significant difference in recovery efficiency among the four adsorbent types was detectable by analysis of variance for either series of experiments (natural viruses: $P = 0.587$, polio: $P = 0.653$). No significant difference was found between mean recovery efficiencies for natural viruses and poliovirus by two-sample t -test ($P = 0.902$).

It should be noted that all or most of the viruses detected in the natural virus mixtures were enteroviruses, which may explain why the recovery rates for these viruses were similar to those for poliovirus type 1. The natural virus mixtures were confirmed as being mostly enteroviruses by "picking" at least one plaque of each observed morphology (about 5-10 plaques per sample) from the assays of the experimental samples and transferring them to fresh BGMK cell cultures for purification and growth and subsequent identification by neutralization tests with enterovirus antisera pools (1). As shown by the results in Table 7, all of the plaque isolates were found to be enteroviruses.

TABLE 7. IDENTIFICATION OF VIRUSES ISOLATED FROM NATURAL VIRUS MIXTURES AND FROM TSM CONCENTRATES OF NATURAL VIRUSES

Sewage Sample	Origin of Isolate	Identification	Number of Isolates
6/13/78	Sewage extract	Poliovirus type 1	2
		Poliovirus type 2	3
		Poliovirus type 3	1
6/13/78	TSM concentrate	Poliovirus type 2	2
8/ 7/78	Sewage extract	Echovirus type 7	1
8/ 7/78	TSM concentrate	Poliovirus type 2	1
8/21/78	Sewage extract	Echovirus type 7	2
		"Enterovirus" ^a	1

^aIsolate neutralized by all Lim-Benesh Melnick enterovirus antiserum pools.

EFFECT OF pH ON ENTERIC VIRUS STABILITY UNDER TSM CONDITIONS

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The stability of representative enteric viruses in tap water and glycine at the various pH extremes encountered in the TSM was investigated in order to determine if this was a factor in the poor virus recoveries that had been obtained. Virus stability experiments in glycine were done at several pH levels in the alkaline range because the pH 11.5 glycine-NaOH used for elution of adsorbed viruses often has a lower pH value after it passes through the filters. Therefore, adsorbed viruses undergoing elution may be exposed to pH 11.5 for only a brief period, but they may be exposed to less alkaline pH values until the entire process is completed, which may be as long as four to five minutes.

In these experiments, one- or two-hundred ml volumes of dechlorinated tap water and glycine were adjusted to the pH values shown in Table 8; and while vigorously mixing magnetically, a small amount of stock virus was added to give an initial concentration of about $2-5 \times 10^3$ PFU/ml. Samples for virus assay were taken at intervals over a total reaction period of 120 min., and they were immediately neutralized by dilution in phosphate buffered saline.

The results of these experiments are summarized in Table 8 in terms of T-90 values; that is, the time in minutes for 90% of the initial viruses to be activated. All of the viruses were stable in tap water at pH 3.0-3.5 and pH 7.0-7.5 with T-90 values well in excess of 120 min. Since the maximum duration of virus exposure to any pH during the TSM procedure is ≤ 120 min., it is unlikely that the poor virus recoveries of the TSM are due to adverse effects of tap water pH. However, all four viruses were affected by alkaline glycine, although there were marked differences in their stability. At pH 9.5, all four viruses were stable (T-90 > 120 min.), but at pH 10.5 poliovirus and MVM remained stable (T-90 > 120 min.), while SV-11 and R-3 became unstable, with T-90 values of 2.8 and 24 minutes, respectively. At pH 11.0 and 11.5 both SV-11 and R-3 were extremely labile with T-90 values of < 1 min. Poliovirus also became unstable at pH 11.0 and 11.5 with T-90 values of 38 and 1.8 min., respectively. Although MVM was the most stable virus tested, it too became unstable at pH 11.5 with a T-90 value of 20 min.

TABLE 8. EFFECT OF pH ON ENTERIC VIRUS STABILITY IN
TAP WATER AND GLYCINE-NaOH

Virus	Time for 90% Virus Inactivation (minutes)					
	Tap Water at pH:		Glycine at pH:			
	3.0-3.5	7.0-7.5	9.5	10.5	11.0	11.5
Minute Virus of Mice	>120	>120	>120	>120	ND ^a	20
Poliovirus type 1	>120	>120	>120	>120	38	1.8
Reovirus type 3	>120	>120	>120	24	<1	<1
Simian Adenovirus SV-11	>120	>120	>120	2.8	<1	<1

^a Not done.

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The results of these experiments indicate that representative viruses from all four enteric virus groups are unstable at pH 11.5 and some are even unstable at less alkaline pH levels. It is apparent that the very poor adenovirus and reovirus recoveries of the TSM can be at least partially, if not entirely, attributed to virus inactivation during elution with pH 11.5 glycine-NaOH. In addition, it is likely that poliovirus and MVM recoveries in the TSM are also reduced due to the adverse effects of the glycine-NaOH eluent.

It should be noted that other workers who have investigated poliovirus stability at pH 11.5 have not observed such rapid losses of infectivity as observed here (24, 27). Although a definitive reason for these differences cannot be provided, they may be due to differences in virus type or strain or in the state of aggregation and purification of the virus stocks. Aggregated viruses are considerably more resistant to inactivation than monodispersed viruses (45).

EFFECTS OF TAP WATER QUALITY ON ENTERIC VIRUS RECOVERY EFFICIENCY

The observation that enterovirus recoveries appeared to vary over time in consecutive experiments (Figure 1) and yet were not significantly different in simultaneous experiments suggested that the variations in the consecutive experiments may be related to temporal changes in water quality. Filter adsorption-elution methods for virus concentration have been shown to be sensitive to differences in water quality, with both soluble (and/or colloidal) and particulate constituents being capable of interfering with virus recovery (14, 15, 16, 23, 25, 36, 38, 40). Because this present study was done with finished tap water having an average turbidity of 0.14 NTU, it was unlikely that particulate matter was responsible for the observed differences in enterovirus recovery efficiency. However, since previous studies have shown that soluble and/or colloidal organic matter can reduce virus recovery by interfering with virus adsorption to filters (14, 15, 16, 35, 36, 40), experiments were done to determine if such interferences were a factor in the poor virus recoveries of this study.

Effect of Tap Water Treatment with Granular Activated Carbon (GAC) on Virus Adsorption and Recovery Efficiency

As one experimental approach to the possibility that soluble and colloidal tap water constituents were interfering with virus adsorption to filters, experiments were done to determine if treatment of the tap water with GAC would improve virus adsorption. Samples of finished tap water were slowly filtered through 10-inch long, 3-inch diameter columns containing about 435 grams of GAC. Volumes of both untreated and GAC-treated tap water were adjusted to pH 3.5 and experimentally contaminated with about 5×10^4 PFU of virus per ml. Twelve- or six-liter volumes were then filtered through 47-mm diameter Cox filters and the filtrates were assayed for viruses. As shown by the results in Table 9, GAC treatment of the tap water produced some improvement in the adsorption efficiency of all three viruses compared to the untreated control tap water. These results suggest that soluble and/or colloidal substances in the tap water that are removable by GAC were indeed interfering with virus adsorption to filters.

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TABLE 9. EFFECT OF TAP WATER TREATMENT WITH GRANULAR
ACTIVATED CARBON ON VIRUS ADSORPTION TO COX FILTERS*

Virus	Virus Adsorption Efficiency (%)	
	Untreated Tap Water	Carbon-Filtered Tap Water
Poliovirus type 1	40	58
Simian Adeno- virus SV-11	22	86
Minute Virus of Mice	68	84
Mean, All Viruses	43	76

* Tap Water Volumes: 12₃ liters for P-1 and MVM, 6 liters for SV-11.
Virus Input: $\approx 5 \times 10^3$ PFU/ml
Adsorption pH: 3.5
Filters: 47-mm dia., 5 and 1 μ m porosity Cox
Results shown are for single trials with each virus.

To further evaluate the effect of GAC treatment on virus recovery efficiency, single-step concentration experiments were done using 12-liter volumes of GAC-treated or untreated tap water containing about 100 PFU of virus (about 1,000 PFU in experiments with CA-9 and MVM). As shown by the results in Table 10, virus recoveries were generally somewhat higher from GAC-treated water than from untreated control water. Analysis of variance showed no significant differences among recovery efficiency for the five enteroviruses from untreated tap water ($P = 0.093$). However, significant differences were found by analysis of variance among recovery efficiencies for the same five enteroviruses from GAC-treated tap water ($P = 0.020$). The difference in virus recovery between GAC- and untreated water was significant in paired t -tests only for BE1 and MVM (BE-1: $P = 0.006$; MVM: $P = 0.003$). While the results for MVM and BE-1 suggest that soluble and/or colloidal constituents in tap water that are removable by GAC significantly interfere with virus recovery, the results with the other viruses indicate that this is not a constant phenomenon. It is possible that the amounts and types of interfering substances in tap water vary over time and that the nature and extent of the interference may also vary with the specific virus type.

GAC treatment of tap water prior to virus adsorption cannot be used as part of a virus concentration procedure for the detection of naturally occurring viruses in water because of the likelihood that viruses would be removed by GAC and thus never reach the adsorbent filter (9). Therefore, alternative methods would have to be used to control or overcome these interferences. However, GAC is certainly useful for further analytical studies on the nature of the soluble and/or colloidal interferences in tap water.

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TABLE 10. EFFECT OF TAP WATER TREATMENT WITH GRANULAR ACTIVATED CARBON ON ENTERIC VIRUS RECOVERY*

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Virus Type	Number of Replicate Trials ⁺	Mean Virus Input PFU	Virus Recovery† percent	
			Untreated Tap Water	GAC-treated Tap Water
P-1	7	180	20 (+ 18)	21 (+ 13)
CB-3	5	211	3.1 (+4.2)	3.8 (+3.8)
CA-9	3	1198	1.9 (+1.9)	9.0 (+ 16)
E-7	5	265	34 (+ 32)	42 (+ 43)
BE-1	6	110	17 (+ 14)	32 (+ 13)
MVM	3	1196	27 (+ 55)	52 (+ 58)

* Tap water volume-12 L; adsorption pH-3.5; filters-47-mm diameter, 5 and 1- μ m porosity Cox filter series; eluent-pH 11.5 glycine-NaOH

Number varies among virus types due to occasional assay failure or virus stock problems resulting in undetectable input.

† Mean percentage recovery of virus input (95 percent confidence interval)

Effect of Tap Water Volume Processed on Virus Recovery Efficiency

Because the results of the previous experiments with GAC indicated that soluble and/or colloidal components in finished tap water may be interfering with virus adsorption to filters, experiments were done to determine if the volume of tap water processed would influence virus recovery efficiency. If substances in tap water were interfering with virus adsorption and/or elution by accumulating on the filters, then presumably there should be a greater interfering effect when processing larger volumes.

In these experiments, 0.5- and 12-liter volumes of dechlorinated tap water were adjusted to pH 3.5 and seeded with the same quantity of stock poliovirus or MVM. Each volume was then filtered through a 47-mm diameter, 1 and 5 μ m porosity Cox filter series, and the adsorbed viruses were then eluted with 15 ml of pH 11.5 glycine-NaOH. As shown by the results in Table 11, virus recoveries were considerably higher from 0.5-liter volumes than from 12-liter volumes by a factor of two or three times for P-1 and MVM, respectively. The differences in virus recovery efficiencies for 0.5 and 12-liter volumes were significant by paired t-test (polio: $P = 0.003$, MVM: $P = 0.002$).

From the results of these experiments, virus recovery efficiencies appear to be dependent on the volume of tap water processed, possibly related to the cumulative effects of soluble and/or colloidal tap water interferences.

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TABLE 11. EFFECT OF TAP WATER VOLUME PROCESSED ON VIRUS RECOVERY EFFICIENCY^a

Virus	Exp. No.	Virus Input (PFU)	Virus Recovery (%) from: ^b	
			0.5 liters	12 liters
Poliovirus type 1	1	22	45	39
	2	71	37	3.2
	3	39	38	54
	4	454	56	18
	5	264	21	0.9
	6	305	50	18
	7	106	28	9.6
	8	535	54	4.0
	9	116	99	48
Mean	--	--	48	22
Minute Virus of Mice	1	1298	54	4
	2	672	>99	48
	3	1338	90	28
Mean	---	--	81	27

^a Virus adsorbent: 47-mm dia., 5 and 1- μ m pore size Cox filter series; elution was with 15 ml of pH 11.5 glycine-NaOH.

^b Paired t-test - Polio: $t=3.617$, $df=8$; $P=0.003$; MVM: $t=14.728$, $df=2$, $P=0.002$.

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STUDIES ON TSM MODIFICATIONS FOR IMPROVED VIRUS RECOVERIES

Studies with TSM and Filterite Adsorbent Filters

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The relatively poor virus recoveries obtained with the TSM in this study were attributed to two major factors: (1) interferences from soluble and/or colloidal substances in tap water, and (2) virus inactivation during elution with pH 11.5 glycine-NaOH. In an effort to overcome these deficiencies, modifications of the TSM were explored. During the course of this study, it was reported that a pleated fiberglass cartridge filter with a large surface area, Filterite, was an effective adsorbent for viruses in water and had the further advantage of processing large volumes at high flow rates (14). In some experiments to be described in this section, it was evaluated either alone or with one or more TSM adsorbents. An additional reason for evaluating another adsorbent filter was that one of the TSM adsorbents (Cox fiberglass-asbestos-epoxy filters) became commercially unavailable due to its asbestos content.

Effect of Magnesium and Aluminum Chloride on Virus Adsorption and Recovery Efficiency

In previous studies, it has been shown that virus adsorption to filters from waters containing soluble and/or colloidal interferences can be enhanced by adding polyvalent cation salts to the water (19, 35). A series of experiments was done to determine if the interfering effects of finished tap water components could be overcome in this manner. In adsorption experiments with SV-11 tap water volumes of 3 or 6 liters were dechlorinated, contaminated with virus and adjusted to the pH levels shown in Table 12. $AlCl_3$ or $MgCl_2$ was added to some of the volumes at the concentrations given in Table 12. The samples were filtered through 47-mm diameter 5- and 1- μ m porosity Cox or 0.45- μ m porosity Filterite filters and the filtrate portions were assayed for unadsorbed viruses. In the absence of added salts virus adsorption to Filterite decreased with increasing pH, but with Cox, no such pattern was observed. Virus adsorption to both filters was markedly improved by 0.0005 M $AlCl_3$ at pH 3.5 and 0.005 M $MgCl_2$ between pH 3.5 and 6.0 (Table 12). In similar experiments with MVM in 0.5-liter tap water volumes and reovirus type 3 in 2-liter tap water volumes at pH 3.5, adsorption efficiency to 47-mm diameter Cox filters was also enhanced by 0.0005 M $AlCl_3$ (Table 13). The results of these experiments indicate that the use of $MgCl_2$ or $AlCl_3$ is an expedient way to overcome the interfering effects of soluble and/or colloidal substances in tap water and thereby enhance virus adsorption. However, further experiments to establish optimum adsorption conditions for all four enteric virus groups could not be done due to the time constraints of this study. This should be an objective of future virus concentrations studies.

Evaluation of Alternative Eluents for Virus Recovery

Because the results of both TSM and virus stability experiments showed that enteric viruses were unstable in pH 11.5 glycine-NaOH, alternative eluents were evaluated for improved recoveries of adsorbed viruses. It has been previously reported that viruses adsorbed to some filter types and certain other surfaces can be eluted with pH 10.5 glycine-NaOH (14, 19) chelating

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TABLE 12. EFFECT OF AlCl_3 AND MgCl_2 ON SV-11 ADSORPTION TO COX AND FILTERITE FILTERS_a

Adsorption Conditions			Virus adsorption (%) to	
pH	Salt	Concentration(M)	Cox	Filterite
3.5	None	--	32	77
3.5	AlCl_3	5×10^{-4}	99	>99
3.5	None	--	31	84
5.0	"	--	73	53
6.0	"	--	50	<1
3.5	MgCl_2	5×10^{-3}	>99	97
5.0	"	--	99	99
6.0	"	--	81	93

Tap Water volumes: All 3 liters except 6 liters for Cox filters with and without AlCl_3 .

Filters: 47-mm diameter, 5 and 1- μm porosity Cox and 0.45 μm porosity Filterite.

Virus input: 3×10^3 PFU/ml.

TABLE 13. EFFECT OF 0.0005 M AlCl_3 ON MVM AND REOVIRUS ADSORPTION TO COX FILTERS

Virus	Virus Adsorption (%)	
	Tap Water (pH 3.5)	Tap Water with AlCl_3 (pH 3.5)
MVM	91	>99
Reo 3	97	>99

Tap water volumes: 500 ml for MVM and 300 ml for Reo 3.

Filters: 47-mm dia., 5 and 1- μm porosity Cox

Virus input: 3×10^2 - 3×10^3 PFU/ml.

agents such as ethylene diamine tetraacetic acid (EDTA) (30, 34) and various alkaline proteinaceous solutions (8, 30, 34). Therefore, two main types of alternative eluents were investigated: glycine-NaOH at pH levels below 11.5 and beef extract both with and without EDTA.

A series of experiments was done with adenovirus SV-11 because of its extreme sensitivity to alkaline pH conditions. In these experiments SV-11 in 0.5- to 6 liter volumes of dechlorinated tap water with added MgCl_2 or AlCl_3 was adsorbed to 47-mm diameter Cox or Filterite filters; the filtrates were assayed for viruses to check for adsorption efficiency. The adsorbed viruses were then eluted with 15-ml volumes of various eluents and the eluates were neutralized with pH 1.5 glycine-HCl and assayed. In some experiments, the eluent was passed through the filters more than once either by

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successive elution with the recovered eluate or by continuous recirculation of the eluent for several minutes with a peristaltic pump. This was done to determine if such additional exposure to the eluent would improve virus recovery.

The results of experiments on SV-11 elution with pH 9.5 glycine-NaOH with and without 0.005 M EDTA are shown in Table 14. SV-11 adsorbed to Cox filters in the presence of $MgCl_2$ or $AlCl_3$ was not efficiently recovered by single elutions with glycine-NaOH or glycine-NaOH-EDTA, although recoveries were somewhat higher when the virus was adsorbed with $MgCl_2$. SV-11 recoveries from Filterite filters by glycine-NaOH with and without EDTA were higher than recoveries from Cox filters, especially when $MgCl_2$ was used to enhance virus adsorption. Because SV-11 recoveries from Filterite were generally higher than those from Cox, only Filterite filters were tested for the effects of repeated elutions with glycine-NaOH and glycine-NaOH-EDTA. Repeated elutions gave somewhat improved virus recoveries under some conditions (Table 14). However, the main factor influencing SV-11 recovery from Filterite was the salt used for enhancing virus adsorption. Recoveries were both higher and more consistent when SV-11 was adsorbed with $MgCl_2$ rather than $AlCl_3$.

Beef extract in 0.05 M glycine at pH 9.5 with and without EDTA was also tested for SV-11 elution efficiency. In initial experiments done with 2.4% beef extract, pH 9.5, with and without EDTA, there was no appreciable difference in virus elution efficiency. However, eluate foaming during multiple elutions made it impossible to obtain precise quantitative results. Therefore, the effect of lower beef extract concentrations on SV-11 elution efficiency was investigated. As shown by the results in Table 15, multiple elutions of SV-11 adsorbed to Cox filters with $AlCl_3$ gave similar recoveries with beef extract concentrations ranging from 0.2 to 2.4%. Furthermore, 0.2% beef extract did not produce appreciable foaming of the eluate.

The elution efficiencies of 0.2% beef extract in glycine with and without 0.005 M EDTA were next compared. SV-11 was adsorbed to Cox and Filterite filters at pH 3.5 with $AlCl_3$ or $MgCl_2$ and then eluted by recirculating the eluent for 5 min. As shown by the results in Table 16, SV-11 was efficiently eluted from both filters by 0.2% beef extract in glycine when $MgCl_2$ was used for virus adsorption. This eluent also efficiently eluted SV-11 from Cox filters when $AlCl_3$ was used for virus adsorption, but elution was less efficient from Filterite. Elution efficiency was actually decreased when EDTA was used.

The results of the experiments on alternative eluents showed that pH 9.5 glycine-NaOH and 0.2% beef extract both with and without EDTA gave improved SV-11 recoveries compared to the pH 11.5 glycine-NaOH eluent of the TSM. Therefore, an experiment was done to compare the efficiency of SV-11 recovery by three different eluents: 0.05 M glycine-NaOH, 0.05 M glycine-NaOH-EDTA and 0.05 M glycine-0.2% beef extract, all at pH 9.5. In this experiment SV-11 in tap water was adsorbed to replicate 47-mm diameter Cox and Filterite filters at pH 3.5 with 0.005 M $MgCl_2$. Each filter was then eluted with one of the three eluents by recirculating for several minutes. As shown by the results in Table 17, maximum virus elution was obtained from Filterite filters using 0.05 M glycine-0.2% beef extract, pH 9.5.

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TABLE 14. ELUTION OF SV-11 FROM COX AND FILTERITE FILTERS
WITH GLYCINE-NaOH AND GLYCINE-NaOH-EDTA, pH 9.5^a

Adsorption Conditions Salt and Conc. (M)		EDTA ^b	Repeated Elutions	Elution efficiency(%) from: Cox Filterite	
AlCl ₃	0.0005	-	-	2.4	14
	"	+	-	3.0	22
MgCl ₂	0.005	-	-	18	64
	"	+	-	22	69
AlCl ₃	0.0005	-	+	ND	40
	"	+	+	ND	22
MgCl ₂	0.005	-	+	ND	52
	"	+	+	ND	69

^a Tap Water Volume: 1.0 liter; Tap Water pH; 3.5.
Virus Input: 3×10^3 PFU/ml; Data are from single trials of each condition.

^b EDTA: 0.005 M
ND = Not Done

TABLE 15. EFFECT OF pH 9.5 BEEF EXTRACT CONCENTRATION ON SV-11
ELUTION FROM COX FILTERS^a

Beef Extract Concentration (%)	Elution Efficiency (%)
0.2	42
0.5	46
1.0	39
2.4 ^b	26

^a Tap Water Volume: 0.5 liters
Filters: 47-mm dia.; 5 and 1- μ m porosity Cox
Virus Input: 3×10^3 PFU/ml
Adsorption conditions: pH 3.5 with 0.0005 M AlCl₃
Eluents were recirculated through the filters.

^b Powdered beef extract at 2.4% corresponds to 3% beef extract made
with beef extract paste.

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TABLE 16. ELUTION OF SV-11 FROM COX AND FILTERITE FILTERS WITH 0.2% BEEF EXTRACT IN GLYCINE OR GLYCINE-EDTA; pH 9.5^a

Adsorption Salt	Conditions and Conc. (M)	EDTA ^b	Virus Recovery (%) from:	
			Cox	Filterite
AlCl ₃	0.0005	-	67	30
MgCl ₂	0.005	-	51	60
"	"	+	41	29

^a Tap Water Volume: 12 liters; adsorption pH: 3.5; 47-mm dia., Cox 5 and 1-μm pore size filters. Virus input: 3 x 10³ PFU/ml; Eluent volume: 15 ml; eluent was recirculated for several min.; results from single trials of each condition.

^b 0.005 M

TABLE 17. COMPARISON OF pH 9.5 GLYCINE-NaOH, GLYCINE-NaOH-EDTA AND GLYCINE-BEEF EXTRACT FOR SV-11 ELUTION

Eluent	Virus Recovery (%) from:	
	Cox	Filterite
glycine-NaOH	34	33
glycine-NaOH-EDTA	40	69
glycine-beef extract	18	96

Adsorption conditions: pH 3.5 with 0.005 M MgCl₂

Glycine: 0.05 M, EDTA: 0.005 M, beef extract: 0.2%.

Virus Input: 3 x 10³ PFU/ml

Results of duplicate experiments.

Virus Reconcentration from Beef Extract by Organic Flocculation

Because viruses eluted with beef extract cannot be further concentrated by adsorption to and elution from filters, organic flocculation (25) was evaluated for reconcentration. Preliminary experiments indicated that a visible floc or precipitate did not form when 0.2% beef extract in 0.05 M glycine was acidified to pH 3.5 and slowly mixed for 30 minutes. An experiment was done to find a suitable beef extract concentration for organic flocculation. In this experiment beef extract solutions in 0.05 M glycine ranging from 0.1 to 3.0% were experimentally contaminated with SV-11, flocculated for 30 minutes and then centrifuged to recover the floc. The supernatant was decanted,

the sediment was resuspended in a small volume of Na_2HPO_4 to a final pH of 7.5, and both fractions were assayed. As shown by the results in Table 18, SV-11 was efficiently flocculated from all of the beef extract solutions tested with recoveries in the sediment ranging from 45 to 77%. It should be noted that a visible floc sediment was not observed at the two lowest beef extract concentrations tested, yet SV-11 was efficiently recovered. On the basis of these results it was decided that beef extract concentrations of 1.5% or higher would be used in subsequent experiments on organic flocculation because it gave efficient virus recoveries and a small but consistent amount of visible floc sediment.

TABLE 18. EFFECT OF BEEF EXTRACT CONCENTRATION ON SV-11 RECOVERY BY ORGANIC FLOCCULATION^a

Beef Extract Conc. (% w/v)	Initial Virus (%) Detected in Supernatant	Detected in Sediment
2.4	1.5	68
2.0	1.4	76
1.5	1.1	71
1.0	1.1	73
0.75	0.1	77
0.5	1.3	61
0.3	0.7	45
0.1	3.5	54

^apH: 3.5; flocculation time: 30 min; Virus Input: 3×10^3 PFU/ml; Results of duplicate experiments.

In order to further investigate virus reconcentration by organic flocculation, an experiment on the effect of flocculation time on SV-11 recovery was done. Replicate solutions of beef extract, 1.5%, in 0.05 M glycine, pH 9.5, were contaminated with SV-11, adjusted to pH 3.5 and flocculated for the time periods shown in Table 19. At each time period a solution was centrifuged to sediment the floc. The supernatant was decanted, the sediment was resuspended in Na_2HPO_4 to pH 7.5 and both fractions were assayed. As shown by the results in Table 19, flocculation time had no effect on either virus removal from the supernatant or recovery in the floc, with recoveries in the range of 47-50% for all flocculation times tested.

TABLE 19. EFFECT OF FLOCCULATION TIME ON SV-11 CONCENTRATION BY ORGANIC FLOCCULATION^a

Flocculation Time (min.)	Initial Virus (%) Detected in Supernatant	Detected in Sediment
<1	<0.4	50
5	0.5	49
15	0.2	43
30	<0.4	47

^a Beef extract, 1.5%, in 0.05 M glycine; flocculated at pH 3.5; Virus Input: 3×10^3 PFU/ml; Results of triplicate experiments.

Experiments were done to determine if SV-11 could also be reconcentrated by organic flocculation of primary eluates. In these experiments, glycine primary eluates obtained by processing 100 gallons of water through each of the primary filters shown in Table 20 were contaminated with SV-11 and supplemented with beef extract to give final concentrations of 1, 1.5 and 2.0%. These samples were then reconcentrated by organic flocculation according to the standard procedure (25). As shown by the results in Table 20, SV-11 was efficiently recovered from all four primary filter eluates by organic flocculation with 1-2% beef extract concentrations, with recoveries ranging from 54 to >99%.

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TABLE 20. ORGANIC FLOCCULATION OF SV-11 IN PRIMARY ELUATES^a

Primary Filter	No. Runs	Beef Extract Conc. (% w/v)	Initial Virus Recovered(%) in: Supernatant Sediment
Cox	2	1.0	1.2 56
	2	1.5	1.5 57
	2	2.0	1.7 54
Millipore	1	1.0	1.9 74
	1	2.0	2.7 90
Carborundum-Cox	1	1.0	1.5 80
	1	2.0	1.5 58
Balston	3	1.0	0.1 86
	3	2.0	0.1 99

^a 100 gallons of tap water processed through primary filters at pH 3.5. Filters eluted with 0.2% beef extract in 0.05 M glycine, pH 9.5. Organic flocculation at pH 3.5 for 30 min. Initial virus input in primary eluates: 5×10^2 PFU/ml.

Alternative elution conditions were also evaluated for some other enteric viruses, although this was done less extensively than for SV-11 due to the time limitations of the project.

The effect of pH on MVM elution from Cox filters with glycine-NaOH was investigated. In this experiment 500-ml volumes of pH 3.5 tap water containing MVM were adsorbed to replicate 47-mm diameter Cox filters. Each filter was eluted with glycine-NaOH at one of the pH levels shown in Table 21. Maximum elution efficiency was obtained at pH 10.5 with virtually complete MVM recovery.

Other eluents including glycine-EDTA and beef extract with and without EDTA were also tested for MVM elution efficiency. In this experiment, MVM was adsorbed to replicate 47-mm diameter Cox filters from 500-ml volumes of pH 3.5 tap water with and without 0.0005 M $AlCl_3$. Each filter was then eluted with one of the eluents shown in Table 22. The results of this experiment (Table 22) indicate that pH 10.5 EDTA and glycine-NaOH or beef extract both

with and without EDTA are effective eluents for MVM adsorbed to Cox filters without added AlCl_3 . However, when MVM is adsorbed to Cox filters with 0.0005 M AlCl_3 , recovery efficiencies were reduced for some eluents; reasonably effective elution was obtained with glycine-NaOH, EDTA and beef extract-EDTA but not with beef extract. The results of these experiments indicate that at least some alternative eluents will efficiently elute MVM but that elution efficiency depends upon adsorption conditions.

Organic flocculation was also examined for MVM reconcentration from beef extract primary eluates. In these experiments 30-ml volumes of 2.4% beef extract experimentally contaminated with about 5×10^2 PFU of MVM were processed by organic flocculation at pH levels of 3.5, 4.0 and 4.5. As shown by the results in Table 23, average MVM recoveries from duplicate trials were only 22, 24 and 14% when flocculated at pH 3.5, 4.0 and 4.5, respectively. No more than 10% of the initial virus was detected in the supernatant at any pH level, indicating that the majority of the viruses were simply not accounted for. Further investigation is needed to determine the reasons for MVM loss and to improve recoveries by organic flocculation.

Explorations of alternative elution and reconcentration methods for reovirus type 3 were also conducted. Two-liter volumes of dechlorinated Chapel Hill tap water were adjusted to pH 3.5, contaminated with 6×10^5 PFU of reovirus and passed through 47-mm Cox 5- μm and 1- μm porosity filter series. Assay of filtrate samples showed that an average 97% of input viruses adsorbed to the filters. After adsorption, filter series were eluted with two successive 7.5-ml volumes of 0.05 M glycine (pH 11.5, 10.5 or 9.5) or 3% beef extract (pH 10.5 or 9.5). Eluates were adjusted to pH 7.5 with 0.05 M glycine, pH 1.5 (for glycine eluates) or 0.2 N HCl (for beef extract eluates) and assayed. The results of these experiments are summarized in Table 24. Efficiency of elution with 0.05 M glycine increased with decreasing eluent pH; glycine at pH 11.5, 10.5 and 9.5 eluted 0.01%, 14% and 45% of adsorbed reovirus, respectively. The proteinaceous medium proved to be a superior eluent to glycine, but also showed better recovery at less alkaline pH; beef extract at pH 10.5 and 9.5 eluted 38% and >99% of adsorbed virus, respectively. Subsequent reconcentration of beef extract eluates (from ~20 ml to ~6 ml) by organic flocculation at pH 3.5 or 4.5 recovered an average 45% of reovirus from the eluates. No marked dependence of reconcentration efficiency on flocculation pH was observed. These results verify that poor reovirus recovery with the TSM can be at least partially explained by reovirus instability in the pH 11.5 glycine-NaOH eluent. The use of less alkaline eluent pH and/or proteinaceous eluent media followed by reconcentration by organic flocculation are alternatives which improve reovirus recovery.

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TABLE 21. EFFECT OF pH ON MVM ELUTION FROM COX FILTERS WITH GLYCINE- NaOH ^a

Glycine- NaOH pH	Elution Efficiency(%)
7.5	38
8.5	29
9.5	59
10.5	99
11.5	37

^a Tap water vol.: 500 ml; adsorption pH: 3.5
47-mm dia., Cox 5 and 1- μm porosity filters
Virus input: 3×10^3 PFU/ml
Results based on single trials of each condition.

TABLE 22. EFFICIENCY OF ALTERNATIVE ELUENTS FOR MVM RECOVERY FROM COX FILTERS^a

Eluent	Elution Efficiency (%) when adsorbed at:	
	pH 3.5	pH 3.5 with AlCl_3
Glycine NaOH :		
pH 11.5	58	68
pH 10.5	78	66
EDTA, pH 10.5	60	52
Beef extract, pH 10	>99	11
Beef extract-EDTA, pH 10	>99	58

^a Adsorption to 47-mm dia., 5 and 1- μm porosity Cox filters from 500-ml volumes of tap water. EDTA: 0.01 M; beef extract: 3%.
Virus input: 3×10^3 PFU/ml
Results based on single trials of each condition.

TABLE 23. MVM RECOVERY FROM 2.4% BEEF EXTRACT BY ORGANIC FLOCCULATION AT DIFFERENT pH LEVELS

pH	Input Virus Detected (%) in:	
	Supernatant	Sediment
3.5	10	22
4.0	2	24
4.5	7	14

Input virus: 2×10^1 PFU/ml of beef extract
Results of duplicate trials.

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TABLE 24. EVALUATION OF MODIFIED ELUTION CONDITIONS FOR REOVIRUS RECOVERY FROM COX FILTERS^a

Adsorption Conditions		Adsorption Efficiency (%)	Eluent	Virus Recovery Efficiency (%)		
pH	Salt Conc. (M)			Primary Elution	Organic Flocculation	Total
3.5	None	--	Glycine-NaOH, pH: 11.5 10.5 9.5	<1 14 44	ND ^b ND ND	<1 14 43
		97%	3% Beef extract, pH: 10.5 9.5	38 >99	35 51	13 49
3.5	AlCl ₃	0.0005	Glycine-NaOH, pH: 9.5	45	ND	45

^a Tap water vol.: 2 l; adsorbent: 47-mm dia., 5 and 1- μ m pore size Cox filters; Virus input: 3×10^2 PFU/ml.

^b Organic flocculation not performed with glycine-NaOH eluates.

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Virus Recoveries with Modified Methods and Negatively Charged Filters

The results of experiments described in the preceding three sections of this report indicated that virus adsorption, elution and reconcentration could be markedly improved by adding aluminum or magnesium chloride to the tap water to enhance virus adsorption, eluting the adsorbed viruses with beef extract and then reconcentrating by organic flocculation. These modified procedures were evaluated in experiments with 100-gallon tap water volumes and low levels of SV-11. SV-11 was adsorbed to Filterite pleated cartridge filters at pH 3.5 with 0.005 M $MgCl_2$. The adsorbed viruses were eluted with 0.2% beef extract 0.05 M glycine, pH 9.5, by recirculating the eluent through the filter for 5 minutes. Additional beef extract was then added to the eluate to give a final 1.5% concentration, and the viruses in the eluate were reconcentrated by organic flocculation at pH 3.5 (25). As shown by the results in Table 25, SV-11 recoveries in nine replicate experiments ranged from 18 to 109% and averaged 53%. Thus, appropriate modifications of the TSM can give marked improvements in virus recovery from tap water.

TABLE 25. CONCENTRATION OF SV-11 FROM
100 GALLONS OF TAP WATER^a

Run No.	Virus Input (PFU)	Virus Recovery(%)
1	219	46
2	129	89
3	123	109
4	40	38
5	431	61
6	40	20
7	40	18
8	171	44
9	229	54
Mean	158	53

^a Primary concentration: adsorption to 0.45 μ m pore size Filterite pleated cartridge at pH 3.5 with 5×10^{-3} M $MgCl_2$; elution with 0.2% beef extract, pH 9.5. Secondary concentration: organic flocculation with 1.5% beef extract at pH 3.5.

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STUDIES WITH ELECTROPOSITIVE FILTERS AS VIRUS ADSORBENTS

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The results of the experiments with the TSM indicated two major deficiencies that resulted in reduced virus recoveries: (1) rapid inactivation of reo-, adeno-, parvo- and polioviruses by pH 11.5 glycine-NaOH, and (2) poor virus adsorption from pH 3.5 tap water due to interfering substances in the finished water. For these reasons, alternative filters that might have different reactivities to enteric viruses and tap water interferences were investigated as virus adsorbents.

Recent electrophoretic studies have established that the microporous filters now employed for concentrating viruses from water are negatively charged throughout the pH range of 2 to 7, with the net negative charge approaching neutrality below pH 2 (26). Electrophoretic studies of viruses indicate that most viruses are also negatively charged near neutral pH and have isoelectric points below pH 7 (6). The fact that both adsorbent filters and viruses are negatively charged near neutral pH may explain the need for acidification or the addition of multivalent cations to obtain efficient virus adsorption from water. Adsorption may be more efficient at pH 3.5 than at pH 7.5 because there is likely to be electrostatic attraction between viruses and filters at the lower pH whereas there is electrostatic repulsion near neutral pH. Near neutral pH, multivalent cations could serve to mediate the adsorption reaction between the two negatively charged surfaces (26, 35).

Microporous filters that are more positively charged in the pH range of most natural and tap waters (pH 5 to 9) would appear to offer advantages over negatively charged filters as virus adsorbents by reducing or eliminating the need for either acid or salt addition to obtain virus adsorption. The different chemical characteristics of these more electropositive filters compared to the conventional electronegative filters might also make the former less susceptible to virus adsorption interference by soluble and/or colloidal constituents of tap water and other waters. Furthermore, if the filters became negatively charged at pH levels somewhat higher than those of tap waters, then it might be possible to elute the adsorbed viruses under moderately alkaline conditions. The two general types of more electropositive filter media studied were Zeta Plus (cellulose-diatomaceous earth-"charged modified" resin) and Seitz (asbestos-cellulose).

Electrophoretic Mobility of Various Filter Media Particles as a Function of pH

As a first step in understanding and comparing the electrochemical surface characteristics of conventional negatively charged adsorbent filters and the more electropositive filters, electrophoretic mobility measurements of various filter media particles were made in solutions ranging in pH from 1.5 to 11.0. The results of these measurements are given in Table 26, and they are also plotted in Figure 2 along with the Filterite data of Kessick and Wagner (26). The electrophoretic mobility of each type of filter medium became more electropositive with decreasing pH, presumably due to protonation of functional groups. Zeta Plus 50S and 60S were the most electropositive filter media, with isoelectric points between pH 5 and 6. Cox AA was somewhat more electronegative with an isoelectric point below pH 3.5. The results for Filterite particles are consistent with those of Kessick and Wagner (26), who

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found that particles of fiberglass as well as cellulosic filters had negative electrophoretic mobilities as low as pH 2. They suggested that filters possessing strongly negative electrophoretic mobilities near neutral pH are predictably poor virus adsorbents in that pH range because the electronegativity of both the filter and virus surfaces would cause them to electrostatically repel each other unless multivalent cations were added to help chemically mediate an adsorption reaction. Because Zeta Plus filters have considerably higher isoelectric points than Cox or Filterite filters and are more electropositive throughout the pH range tested, it is possible that they would adsorb negatively charged viruses at considerably higher pH levels than the electropositive filters without having to add multivalent cations. Cox filters, which are electropositive up to about pH 3.5, are expected to be intermediate between the more electronegative Filterite filters and the more electropositive Zeta Plus filters in terms of their ability to adsorb viruses.

TABLE 26. ELECTROPHORETIC MOBILITY OF VARIOUS FILTER MEDIA

pH	Electrophoretic Mobility ($\mu\text{m}/\text{sec}/\text{volt}/\text{cm}$) ^a			
	Filterite	Cox	Zeta Plus 50S	Zeta Plus 60S
1.5	-0.60	+0.70	ND ^b	ND
3.0	-1.80	+0.30	+1.02	+1.10
5.0	-2.40	-1.00	+0.26	+0.46
7.0	-2.60	-2.00	-0.74	-0.52
9.0	ND	ND	-1.70	-1.18
11.0	ND	ND	-1.51	-1.67

^aIonic strength = 0.05

^bND = not done.

It should be mentioned that measurements of the electrophoretic mobilities of Seitz filter particles were also made. The mobility curve was similar in shape to the Zeta Plus curves, having an isoelectric point near pH 7. However, in view of the fact that chrysotile asbestos particles are isoelectric near pH 12 (32), the isoelectric point determination for the Seitz filters may be low and may be an artifact of the particle preparation procedure. Chrysotile asbestos is a hollow fiber structure with a strongly electropositive nature due to the unique geometrical and spatial characteristics of its charged functional groups. Strongly electropositive magnesium hydroxide functional groups compose the outer, exposed surface of the fiber, whereas the strongly electronegative silicates are confined to inner, unexposed surfaces. A possible explanation for a lower than expected isoelectric point, is that the blending required for particle preparation may have sheared the fibers, exposing highly negative silicate groups which were previously unexposed, and thereby lowering the net surface charge of the particle.

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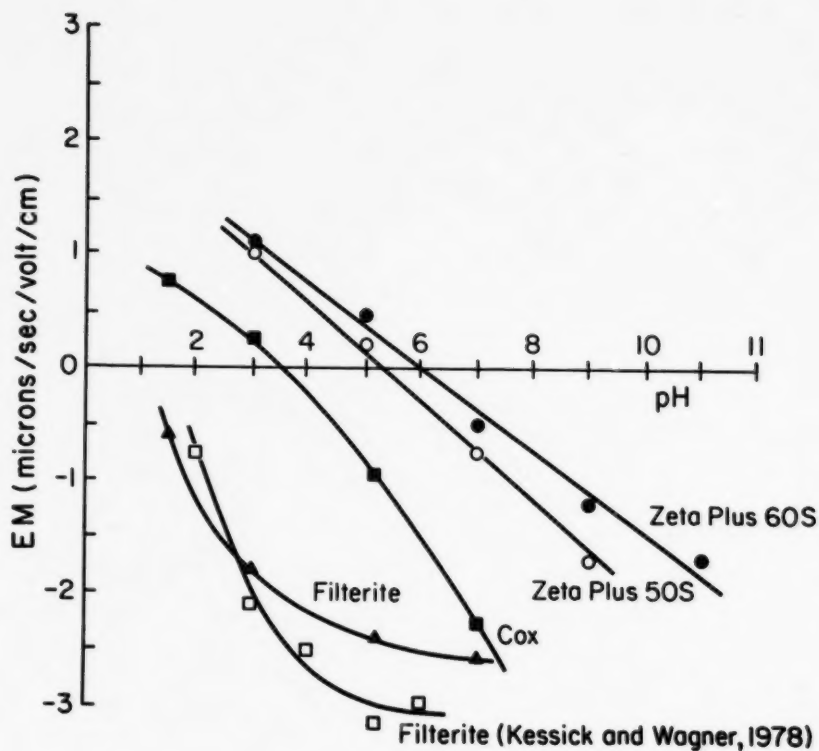


Figure 2. Electrophoretic mobility of filter media as a function of pH

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Effect of pH on Poliovirus Adsorption to Various Filters

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As an initial step in evaluating positively charged filters for concentrating viruses from water, the effect of tap water pH on poliovirus adsorption was determined. Five hundred-ml volumes of dechlorinated tap water were adjusted to the pH levels shown in Table 27, seeded with about 10,000 PFU of poliovirus per ml and assayed for initial virus concentration. These volumes were filtered through the indicated media at fluxes of about 10 ml per cm² per min., and the filtrates were assayed for viruses. Two successive 7.5-ml volumes of pH 11.5 glycine-NaOH were passed through each filter to elute adsorbed viruses. The total 15-ml eluate was quickly adjusted to pH 7.4 and assayed for viruses. Although poliovirus was efficiently adsorbed to the negatively charged Filterite and Cox filters at pH 3.5 and was efficiently eluted with pH 11.5 glycine-NaOH, virus adsorption, and hence, recovery efficiencies, were greatly reduced at pH 5.5 and 7.5 (Table 27). It should be noted that the more electropositive Cox filters adsorbed approximately twice as much virus at pH 5.5 and 7.5 as the more electronegative Filterite filters. Zeta Plus 50S filters efficiently adsorbed viruses between pH 5.5 and 7.5, but not at the higher or lower pH levels tested, while Zeta Plus 60S filters efficiently adsorbed viruses between pH 3.5 and 6.0, but were somewhat less effective at the higher pH levels tested. Figure 3 clearly shows the superiority of Zeta Plus 50S over Cox and Filterite filters as a virus adsorbent in the pH range of most natural and tap waters. For both Zeta Plus 50S and 60S filters, the majority of adsorbed viruses were recovered by elution with pH 11.5 glycine-NaOH. Seitz filters adsorbed virtually 100% of poliovirus from tap water at pH 3.5 to 9, but the adsorbed viruses could not be eluted with pH 11.5 glycine-NaOH. Because Zeta Plus 50S and 60S and Seitz S filters are capable of efficiently adsorbing polioviruses at considerably higher pH levels than the more electronegative filters, such as Cox and Filterite, they may be capable of adsorbing viruses directly from tap water at ambient pH levels without having to add multivalent cations.

For the results summarized in Table 27, statistical comparisons of adsorption efficiencies for pairs of filter media at various pH levels were made using the raw data of the experiments. As shown in Table 28, statistical comparisons of adsorption efficiencies between filter pairs shows that the Zeta Plus filters are more effective virus adsorbents at pH 5.5 and 7.5 than Cox filters. No significant differences in virus adsorption were observed between Cox at pH 3.5 and Zeta Plus 60S at pH 6.0 or Zeta Plus 50S at pH 7.5.

Effect of pH on the Adsorption of Other Viruses to Zeta Plus Filters

A series of experiments was done to determine the effect of tap water pH on MVM adsorption to Zeta Plus 30S, 50S and 60S filters. Tap water contaminated with MVM was adjusted to pH 3.5, 5.5 and 7.5. For each pH level, 500-ml volumes were filtered through 47-mm diameter Zeta Plus filters, and the filtrates were assayed for unadsorbed viruses. Adsorbed viruses were eluted with pH 11.5 glycine-NaOH, and the eluates were neutralized and assayed for viruses. As shown by the results in Table 29, the Zeta Plus filters efficiently adsorbed MVM at the three pH levels tested, although adsorption efficiency was somewhat reduced at pH 7.5. Furthermore, pH 11.5 glycine was reasonably effective in eluting adsorbed viruses with efficiencies ranging from 36 to 76 percent.

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TABLE 27. EFFECT OF TAP WATER pH ON POLIOVIRUS ADSORPTION TO AND ELUTION FROM VARIOUS FILTER MEDIA^a

Adsorption pH	Percentage of Total Input Virus Adsorbed				
	Filterite	Cox	Zeta Plus 50S	Zeta Plus 60S	Seitz
3.5	93	99	63	>99	>99
5.5	17	34	98	>99	>99
6.0	ND ^b	ND	ND	>99	ND
7.0	ND	ND	99	72	>99
7.5	7	18	99	61	>99
8.5	ND	ND	26	ND	ND
9.0	ND	ND	ND	ND	>99

	Percentage of Total Input Virus Recovered in Eluate				
	Filterite	Cox	Zeta Plus 50S	Zeta Plus 60S	Seitz
3.5	66	50	38	77	0
5.5	4	9	69	73	0
6.0	ND	ND	ND	60	ND
7.0	ND	ND	63	45	0
7.5	3	8	67	42	0
8.5	ND	ND	1	ND	ND
9.0	ND	ND	ND	ND	0

^aTap water vol. = 500 ml; filter dia. = 47-mm; eluent: 0.05 M glycine-NaOH,

^bND = not done.

Results from duplicate or triplicate runs for each condition.

TABLE 28. STATISTICAL COMPARISON OF POLIOVIRUS ADSORPTION EFFICIENCIES FOR ZETA PLUS AND COX FILTERS

Comparison Between:			Significant ^a Difference	Standard Deviate	P Value
60S @ pH 5.5	and	Cox @ pH 5.5	yes	6.46	<.0001
60S @ pH 7.5	and	Cox @ pH 7.5	yes	4.41	<.0001
50S @ pH 5.5	and	Cox @ pH 5.5	yes	4.19	<.0001
50S @ pH 7.5	and	Cox @ pH 7.5	yes	7.83	<.0001
60S @ pH 6.0	and	Cox @ pH 3.5	no	0	
50S @ pH 7.5	and	Cox @ pH 3.5	no	0	

^a Significance level = 95%.

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TABLE 29. EFFECT OF pH ON MVM ADSORPTION TO AND ELUTION FROM ZETA PLUS FILTERS^a

pH	Virus Adsorption (%)			Elution Efficiency (%)		
	30S	50S	60S	30S	50S	60S
3.5	>99	>99	>99	ND ^b	41	36
5.5	>99	>99	99	ND	44	56
7.5	76	91	91	70	55	71

^aTap water vol.: 500 ml; eluent: 0.05 M glycine-NaOH, pH 11.5; virus input: 10^4 PFU/ml.

^bND = not done.

The effect of pH on SV-11 adsorption to Zeta Plus 50S and 60S was also examined. Experimental procedures were generally similar to those described above for MVM. As shown by the results in Table 30, SV-11 was adsorbed with an efficiency of >99% by both filters between pH 5.0 and 7.5. Adsorption efficiency of 60S decreased to about 80% at pH 3.0 and 4.0. Zeta Plus 50S adsorption efficiency was additionally tested only at pH 4.5, where it was still >99%.

TABLE 30. EFFECT OF pH ON SV-11 ADSORPTION TO ZETA PLUS 50S AND 60S FILTERS^a

pH	Virus Adsorption (%) to:	
	50S	60S
3.0	ND ^b	81
4.0	ND	80
4.5	>99	98
5.0	>99	>99
5.5	>99	>99
6.5	>99	>99
7.5	>99	>99

^aTap water vol: 500 ml; Filter dia., = 47-mm; Input virus: 3×10^3 PFU/ml.

^bND=Not done.

Results from single trials for each condition.

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Poliovirus Elution from Positively Charged Filters

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Positively charged Zeta Plus 50S and 60S and Seitz S filters were further studied with respect to the conditions for eluting adsorbed viruses. Although the previous experiments showed that the majority of viruses adsorbed to both Zeta Plus filter types could be recovered by elution with glycine-NaOH at pH 11.5, these filters were tested to determine if efficient virus elution with glycine-NaOH could be obtained at lower pH levels. Elution at pH levels lower than 11.5 is desirable because many enteric viruses are unstable under such extremely alkaline conditions. In these experiments 500-ml volumes of dechlorinated tap water seeded with about 10,000 PFU of poliovirus per ml were filtered through 47-mm diameter Zeta Plus 50S and 60S filters at pH 7.0 and 6.0, respectively, for virus adsorption. These pH levels were selected to assure at least 99% virus adsorption, and the filtrates were assayed for viruses to confirm that this adsorption efficiency has been achieved. The adsorbed viruses were then eluted with two successive 7.5-ml volumes of 0.05 M glycine buffer at either pH 11.5, 10.5 or 9.5, and the eluates were quickly adjusted to pH 7.4 and assayed for viruses. As shown by the results in Table 31, greater than 60% poliovirus elution efficiency was obtained at all pH levels tested, including pH 9.5 where virus recoveries averaged 88% and 74% for Zeta Plus 50S and 60S, respectively. Statistical evaluation of these data by t-test showed no significant difference in elution efficiency between pH 9.5, 10.5, and 11.5 (P greater than 0.10 in all cases). However, it should be noted that the lowest elution efficiency occurred with pH 11.5 glycine-NaOH, which may reflect some virus inactivation at this extreme pH. The ability to obtain efficient virus elution under moderately alkaline conditions, such as pH 9.5-10, minimizes possibilities of virus inactivation by hydroxide alkalinity and allows for longer contact times between filter and eluent.

TABLE 31. EFFECT OF pH ON POLIOVIRUS ELUTION FROM ZETA PLUS FILTERS WITH GLYCINE-NaOH^a

Filter	Elution Efficiency (%) at pH:			
	11.5	10.5	10.0	9.5
50S	65	70	ND ^b	88
60S	67	82	79	74

^aTap water vol. = 500 ml; filter diam = 47-mm; elution vol. = 15 ml; Adsorption pH = 7.0 for 50S 6.0 for 60S; Input virus = 10⁴ PFU/ml.

^bND=Not done.

Results from duplicate trials for each condition.

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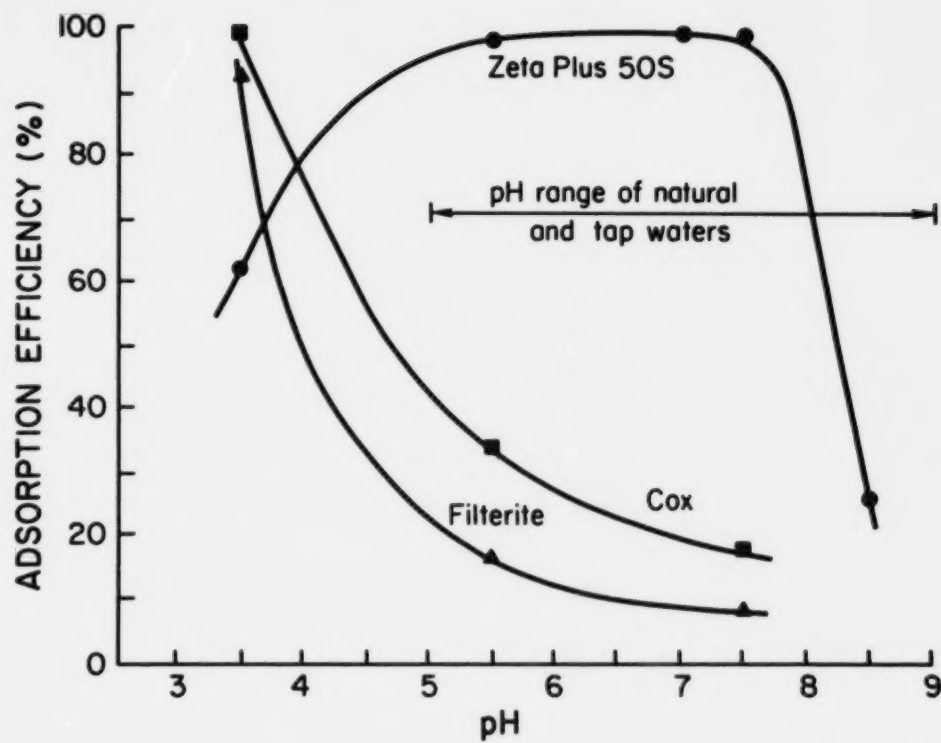
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Figure 3. Poliovirus adsorption to filter media as a function of pH

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As previously noted, elution of poliovirus from Seitz filters was not possible using glycine-NaOH at pH 11.5. This would be expected if Seitz filters are as strongly electropositive as believed (isoelectric point at pH 11-12) and if elution by strongly basic buffers relies on electrostatic repulsion to overcome the forces of adsorption, because no net charge reversal of the filter would take place at pH 11.5. In further investigations it was found that 3% beef extract at pH 9 was an effective eluent for Seitz filters. In these experiments poliovirus was adsorbed to Seitz filters at ambient tap water pH levels (7.0-7.5) and eluted with various eluents using the general procedures previously described for the elution experiments with Zeta Plus filters. The results of these experiments (Table 32) indicate that greater than 70% elution efficiency was possible with 3% beef extract at pH 9. Elution efficiency was lower when 3% beef extract was used at pH 7, and the lowest elution efficiencies were obtained with pH 9 and 11.5 glycine-NaOH. This demonstration of effective virus elution from Seitz filters, coupled with their extremely wide pH range for efficient virus adsorption, further establishes the usefulness of highly electropositive filters in concentrating viruses from tap water at ambient pH levels.

TABLE 32. POLIOVIRUS ELUTION FROM SEITZ S FILTERS^a

Eluent	Elution Efficiency (%)
3% beef extract, pH 9	73
3% beef extract, pH 7	30
Glycine-NaOH, pH 9	1
Glycine-NaOH, pH 11.5	<1

^aTap water vol. = 500 ml; filter dia. = 47-mm;
adsorption at ambient tap water pH (7.0-7.5);
elution vol. = 15 ml; Input virus = 10⁴ PFU/ml.

Results from single trials for each condition.

Elution of Other Viruses from Zeta Plus Filters

Glycine-NaOH and beef extract at different pH levels were tested for the elution of MVM adsorbed to Zeta Plus 50S filters. In these experiments, 500-ml volumes of MVM-contaminated, pH 7.5 tap water were filtered through 47-mm diameter Zeta Plus 50S filters and the filtrates were assayed to check for efficient virus adsorption. The adsorbed viruses were eluted with glycine-NaOH or 2.4% beef extract at the pH levels shown in Table 33. Glycine-NaOH eluted MVM with efficiencies of 60, 70 and 55% at pH 9.5, 10.5 and 11.5, respectively. Beef extract was an even more efficient eluent, giving recoveries ranging from 91% at pH 7.0 to 75% at pH 10.0. Similar experiments were done on MVM elution from Zeta Plus 30S filters with glycine and beef extract with and without EDTA. As shown by the results in Table 34, essentially complete elution was obtained with all beef extract eluents and with pH 10.5 glycine-EDTA, with somewhat reduced elution efficiencies by pH 10.5 and 11.5 glycine-NaOH.

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TABLE 33. EFFECT OF pH ON MVM ELUTION FROM ZETA PLUS 50S WITH GLYCINE-NaOH AND 2.4% BEEF EXTRACT^a

Eluent and pH	Elution Efficiency (%)
Glycine-NaOH, pH:	
9.5	60
10.5	70
11.5	55
Beef Extract, pH:	
7.0	91
8.0	78
9.0	74
10.0	75

^aTap water vol.: 500 ml; adsorption pH: 7.5. Filter dia.: 47-mm; Input virus 5×10^3 PFU/ml.

Results for single trials of each condition.

TABLE 34. MVM ELUTION FROM ZETA PLUS 30A WITH VARIOUS ELUENTS^a

Eluent and pH	Elution Efficiency (%)
Glycine-NaOH, pH:	
11.5	70
10.5	63
Glycine-NaOH-EDTA, pH 10.5	100
Beef extract, pH:	
10	100
7	100
Beef extract-EDTA, pH 10	100

^aTap water vol.: 500 ml; Filter dia.: 47-mm; Adsorption at ambient pH levels (7.0-7.1); Glycine: 0.05 M; EDTA: 0.01 M; Beef extract: 2.4%; Input virus: 5×10^3 PFU/ml. Results for single trials of each condition.

The adenovirus SV-11 was less effectively eluted from Zeta Plus filters than either poliovirus or MVM. Three eluents were tested in experiments similar to those with MVM: glycine, glycine-EDTA and beef extract. As shown by the results in Table 35, SV-11 was recovered more effectively from Zeta Plus 60S than from 50S filters. All three eluent types recovered about one-fourth of the viruses from 60S filters but less than 10% of the viruses from 50S. In addition, it should be noted that elution efficiencies from both filters were highly variable from one experiment to another. The reason(s) for such poor and inconsistent SV-11 elution from Zeta Plus filters could not be investigated in this study and further research should be done in future studies.

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TABLE 35. SV-11 ELUTION FROM ZETA PLUS 50S AND 60S FILTERS BY DIFFERENT ELUENTS^a

Eluent and pH	Elution Efficiency (%) from:			
	50S		60S	
	Mean	Range	Mean	Range
Glycine, pH:				
3.0	6.0	1-57	29	1-84
10.0	2.0	1-3.7	25	8.4-34
Glycine-EDTA, pH 10	1.0	-	19	11-26
Beef extract, pH:				
7	5.5	-	ND ^b	
9	ND	-	24	18-28
10	6.8	5.6-8.5	4.0	2.6-5.5

^aTap water vol.: 500 ml.; adsorption pH: 4.5; Filter dia.: 47-mm; eluents: 0.05 M glycine, 0.05 M glycine-EDTA (0.1-0.5 M), and 3% beef extract; Input virus: 3 x 10³ PFU/ml.

^bNot done.

Reconcentration of Poliovirus from Primary Eluates

Reconcentration of viruses in primary eluates obtained by processing 378-L (100 gal) of tap water was evaluated for Zeta Plus filters. Primary eluates were obtained by filtering 378 L of dechlorinated tap water at pH 6 through a 267-mm diameter Zeta Plus 60S filter disc, eluting with 1 liter of glycine at pH 10, and neutralizing to pH 7.5. This primary eluate was then subdivided into smaller volumes and seeded with poliovirus. Both Zeta Plus 50S and 60S filters were tested for virus reconcentration efficiency at two adsorption pH levels and two elution pH levels, and the results of these experiments are given in Table 36. In the reconcentration step, greater than 90% virus adsorption efficiency was obtained at pH 6.5 and 5.0, respectively, for Zeta Plus 50S and 60S. These are only slightly lower than the adsorption pH levels used in primary virus concentration. The slightly lower pH required for virus adsorption in the reconcentration step may be due to the higher content of interfering tap water constituents in the primary eluates compared to tap water. Efficient elution of adsorbed viruses was obtained with pH 10 as well as pH 11.5 glycine-NaOH. Thus, with only moderate pH adjustments and no addition of multivalent cation salts, both Zeta Plus filter media were capable of efficiently reconcentrating polioviruses from primary eluates.

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TABLE 36. POLIOVIRUS CONCENTRATION FROM PRIMARY ELUATE
(RECONCENTRATION)^a

Filter	Adsorption		Recovery Efficiency (%) ^b	
	Adsorption pH	Efficiency (%)	pH 10	pH 11.5
Zeta Plus	7.5	53	75	40
50S	6.5	98	75	85
Zeta Plus	6.0	85	110	85
60S	5.0	92	110	110

^aPrimary eluate vol. = 500 ml; Filter dia. = 47-mm; Virus input: 3×10^3 PFU/ml.

^bElution with 15 ml of glycine-NaOH.

Concentration of Low Virus Levels Using Zeta Plus Filters

Large volume experiments with low input virus levels were done in which 12-l volumes of tap water were processed through 47-mm diameter Zeta Plus 50S filters at ambient pH levels, ranging from pH 7.0-7.5. Processing 12-l through a 47-mm diameter filter is approximately equivalent in hydraulic loading to processing 378 l through the 267-mm diameter filters normally employed for large-scale primary virus concentration. Adsorbed viruses were eluted with 15 ml of pH 10 glycine-NaOH, and the entire eluate was assayed for viruses. As shown in Table 37, the mean virus recovery for six experiments was 64% and the median virus recovery was 56%. In similar experiments with Seitz filters, virus recovery was 28% when adsorbed viruses were eluted with 3% beef extract at pH 9.0. This recovery from Seitz filters at pH 9.0 is lower than that previously obtained. The reasons for this difference in recovery efficiency were not further investigated and cannot be adequately explained.

TABLE 37. RECOVERY OF LOW LEVELS OF POLIOVIRUS FROM 12-LITER
VOLUMES OF TAP WATER USING ZETA PLUS 50S FILTERS^a

Exp. No.	Amount of Virus (PFU)		Volume Concen- tration Factor	Input Virus Recovered (%)
	Input	Recovered		
1	22	14	460	64
2	53	30	460	57
3	97	43	460	44
4	217	119	475	55
5	33	44	370	133
6	144	46	480	32
Mean	-	-	-	64
Median	-	-	-	56

^aFilter dia. = 47-mm; adsorption at ambient tap water pH (7.0-7.5); elution with pH 10.0 glycine-NaOH, 15 ml.

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To demonstrate the applicability of Zeta Plus 50S filters to a more realistic situation involving 378-l (100-gallon) volumes of tap water, 267-mm diameter filter disks were used in the primary step of a two-stage flow-through adsorption-elution experiment. Low levels of monodispersed poliovirus were seeded by a fluid proportioner into 378-l volumes of tap water, and concentrated by two-stage filter adsorption-elution procedures to final volumes of about 25 ml. Two Cox filters, one under adsorption-elution conditions identical to those for the Zeta Plus 50S filters and another under conditions specified in the 14th ed. of Standard Methods (1), were utilized in simultaneous experiments as controls. Virus recoveries were much greater with the Zeta Plus filters than with either of the Cox filters -- 20 and 25% recovery with Zeta Plus filters and only 2 and 4% recovery with Cox filters (Table 38). These results further demonstrate the superiority of Zeta Plus filters as virus adsorbents under certain conditions. In addition, the concentration procedure was much simpler when Zeta Plus filters were used because the need for continuously adding controlled amounts of acid and/or salts was eliminated. In another set of experiments, the recoveries of low levels of poliovirus adsorbed to Cox and Zeta Plus 50S and 30S filters from 100-gallon volumes of tap water were compared for the various eluents shown in Table 39. Compared to the 5 percent recovery obtained with TSM conditions and Cox filters, recoveries from Zeta Plus 50S and 30S with glycine-NaOH-EDTA were 69 and 62 percent, respectively, and with pH 10 beef extract, recovery from 50S was virtually complete. These results further demonstrate the superiority of Zeta Plus over Cox filters as poliovirus adsorbents.

TABLE 38. COMPARISON OF COX AND ZETA PLUS FILTERS FOR TWO-STAGE CONCENTRATION OF POLIOVIRUS FROM 378-LITER VOLUMES OF TAP WATER

Adsorbent Filters ^a	pH Conditions				Amount of Virus (PFU)		Virus Reco- vered (%)
	Primary Conc.		Reconcentration				
	Adsorp- tion	Elu- tion	Adsorp- tion-	Elu- tion ^b	Input	Recovered	
Zeta Plus 50S	7.1	10	6	10	413	103	25
Zeta Plus 50S	7.1	10	6	10	426	85	20
Cox	7.1	10	6	10	407	8	2
Cox ^c	3.5	11.5	3.5 ^d	11.5	305	13	4

^aPrimary and secondary adsorbents were 267 and 47-mm diameter, respectively. Zeta plus primary adsorbent was preceded by 1 μ m porosity polypropylene prefilter.

^bElution with 0.05 M glycine-NaOH.

^cConditions specified in Standard Methods, 14th edition (1).

^dWith 0.0005 M $AlCl_3$.

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TABLE 39. COMPARISON OF VARIOUS ELUENTS FOR POLIOVIRUS RECOVERY FROM 100-GALLON TAP WATER VOLUMES WITH COX AND ZETA PLUS FILTERS^a

Eluent and pH	Virus Recovery (%) from:			
	Cox ^a	Cox ^b	Zeta Plus ^c 50S	30S
Glycine-NaOH, pH:				
11.5	5	4	ND ^d	ND
10.5	7	16	18	ND
Glycine-NaOH-EDTA, pH 10.5	21	18	69	62
Beef extract-EDTA ^e , pH 10 ^f	35	ND	99	ND

^aPrimary adsorption at pH 3.5; secondary adsorption at pH 3.5 with 0.0005 M AlCl₃.

^bPrimary adsorption at pH 3.5 with 0.0005 M AlCl₃; secondary adsorption as in footnote a.

^cPrimary adsorption at ambient pH (7.0-7.5); Secondary adsorption at pH 6.0.

^dND = Not Done.

^eEDTA = 0.01 M

^fBeef extract eluates reconcentrated by organic flocculation.

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The results of these studies show that Zeta Plus and perhaps other electropositive filters have considerable promise as improved adsorbents for concentrating viruses from large volumes of tap water. These filters are more effective adsorbents than electronegative filters in the pH range of natural and tap waters. The ability of Zeta Plus filters to efficiently adsorb viruses at ambient pH levels up to 7.5 makes chemical modification of many waters unnecessary, and thereby eliminates the need for cumbersome and expensive chemical additive systems. Because adsorbed viruses can be eluted from Zeta Plus filters with pH 9.5 to 10.0 glycine-NaOH or beef extract, the opportunities for virus inactivation are reduced compared to those that occur when using pH 11.5 glycine-NaOH.

The results of this study also suggest the electropositive filters may be less susceptible to certain chemical interferences in tap and other waters because they concentrated poliovirus from tap water more effectively than did a representative electronegative TSM filter (Cox). Another advantage of electropositive filters related to water quality is the elimination of precipitation and resultant filter clogging that may occur when acidifying or adding multivalent cation salts to primary eluates.

While the results of this study indicate that electropositive Zeta Plus filters show considerable promise as improved virus adsorbents, additional studies are needed to determine if they will be effective with other waters and virus types.

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TABLE A1. ENTERIC VIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE TSM: POLIOVIRUS TYPE 1^a

Date	Balston				Cox				Carborundum-Cox				Millipore			
	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Tap Water Vol. (gal)
6/24/76	100	77.1	9.0	100	77.1	49.5	100	77.1	66.7	86.5	100	77.1	68.2	88.4	100	77.1
7/1/76	100	81.0	24.8	100	81.1	9.0	100	83.6	3.4	4.1	100	81.0	5.6	6.9	100	81.0
7/8/76	100	66.7	9.0	100	67.2	10.1	100	67.2	18.0	26.8	100	66.7	15.8	23.7	100	66.7
7/15/76	100	45.6	6.8	100	45.5	<1.1	83	37.6	9.0	23.9	100	45.5	6.8	14.9	100	45.5
7/20/76	103	78.1	10.1	100	75.7	15.8	100	75.8	15.8	20.8	100	75.6	9.0	11.9	100	75.6
7/22/76	100	57.4	13.5	100	57.4	12.4	100	57.9	6.8	11.7	100	57.3	6.8	11.9	100	57.3
7/28/76	100	50.4	12.4	103	51.7	16.9	100	50.5	29.3	58.0	100	50.4	32.6	64.7	100	50.4
7/30/76	100	57.0	21.4	103	59.0	33.8	100	56.9	38.3	67.3	100	56.9	20.3	35.7	100	56.9
8/12/76	101	38.8	7.9	100	38.4	10.1	100	38.4	15.8	41.1	100	38.3	15.8	41.2	100	38.3
8/18/76	100	147.3	<1.1	101	148.3	<1.1	100	147.3	1.1	0.7	107	158.3	3.4	2.1	107	158.3
9/9/76	95	93.7	4.5	101	100.4	6.8	100	98.9	37.1	37.5	85	84.2	13.5	16.0	101	43.2
9/16/76	101	43.2	1.1	101	43.2	<1.1	101	43.2	<1.1	<2.5	101	43.2	1.1	2.5	101	43.2
9/23/76	101	58.7	<1.1	101	58.7	<1.1	84	48.7	<1.1	<2.2	101	58.7	<1.1	<1.9	101	58.7
Avg. ^b	100	68.8	15.1 (±7.1)	101	69.5	19.7 (±12.8)	98	67.9	29.1 (±16.8)	29.1 (±16.8)	100	68.7	24.6 (±16.1)	24.6 (±16.1)	100	68.7

^a"Less-Than" values assumed 0% for calculations^bAverage (95% confidence interval)Anova: F = 0.915, v₁ = 3, v₂ = 48; p = 0.441

TABLE A2. ENTERIC VIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE TSM: COXSACKIEVIRUS TYPE B3^a

Date	Balston				Cox				Carborundum-Cox				Millipore			
	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)
		Input	Recov.			Input	Recov.			Input	Recov.			Input	Recov.	
10/13/76	101	77.1	4.5	5.8	101	77.1	3.4	4.4	100	77.1	3.4	4.4	101	77.1	2.3	3.0
10/19/76	103	55.1	2.3	4.2	99	53.1	6.8	12.8	101	53.9	2.3	4.3	101	53.9	1.1	2.0
10/21/76	100	88.9	1.1	1.2	100	88.9	<1.1	<1.2	100	88.8	1.1	1.2	100	88.9	<1.1	<1.2
10/26/76	100	63.9	<1.1	<1.7	100	63.8	<1.1	<1.7	100	63.8	<1.1	<1.7	100	63.8	<1.1	<1.7
10/28/76	100	52.3	1.1	2.1	100	52.4	2.3	4.4	100	52.3	1.1	2.1	100	52.3	<1.1	<2.1
11/1/76	100	23.9	1.1	4.6	100	23.9	1.1	4.6	100	23.9	1.1	4.6	100	23.9	<1.1	<4.6
11/3/76	100	35.9	<1.1	<3.1	100	35.8	<1.1	<3.1	100	35.8	1.1	3.1	100	35.8	<1.1	<3.1
Avg. ^b	100	56.7		2.6 (±2.2)	100	56.4		3.7 (±4.2)	100	56.5		2.8 (±1.6)	100	56.5		0.7 (±1.2)

^aLess-Than" values assumed 0% for calculations

^bAverage (95% confidence interval)

Anova: F = 1.449, $v_1 = 3$, $v_2 = 24$, $p = 0.253$

TABLE A3. ENTERIC VIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE ISM: ECHOVIRUS TYPE 7^a

Date	Ballston				Cox				Carborundum-Cox				Millipore			
	Tap Water Vol. (gal)	Virus Input	(PFU) Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input	(PFU) Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input	(PFU) Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input	(PFU) Recov. (%)	Virus Recov. (%)
1/28/77	100	48.4	3.6	7.4	100	48.4	7.5	15.5	100	48.3	3.5	7.2	100	48.4	6.0	12.4
2/2/77	100	117.3	1.3	1.1	100	117.3	12.6	10.7	100	117.1	<1.1	<0.9	100	117.3	4.5	3.8
2/4/77	100	102.9	1.2	1.2	100	103.1	1.2	1.2	100	102.9	2.3	2.2	100	103.0	<1.1	<1.1
2/10/77	91	108.6	11.0	10.1	100	120.1	5.5	4.6	42	50.7	9.9	19.5	34	41.3	13.2	32.0
2/15/77	100	102.2	1.2	1.2	100	102.2	2.3	2.2	100	102.4	13.7	13.4	66	67.2	5.7	8.5
2/18/77	100	105.0	<1.1	<1.0	100	104.9	4.6	4.4	86	90.0	2.3	2.6	67	69.7	3.5	5.0
2/22/77	100	102.8	3.4	3.3	100	102.8	4.6	4.5	100	103.0	13.6	13.2	65	66.8	6.9	10.9
2/24/77	100	127.1	<1.1	<0.9	100	127.1	2.3	1.8	100	127.1	9.0	7.1	100	127.1	10.3	18.1
3/1/77	100	177.9	1.2	0.7	100	178.0	8.0	4.5	100	178.0	21.6	12.1	46	82.0	12.6	15.4
3/8/77	102	114.0	<1.1	<1.0	100	112.2	3.3	2.9	61	68.5	9.9	14.4	39	43.2	2.2	5.1
Avg. ^b	99	110.6		2.5 (±2.5)	100	111.6		5.2 (±3.2)	89	98.8		9.2 (±4.5)	72	76.6		10.1 (±6.4)

^a"Less-Than" values assumed 0% for calculations

^bAverage (95% confidence interval)

Anova: $F = 3.274$, $v_1 = 3$, $v_2 = 36$; $P = 0.032$

Anova: (Runs in which any filter clogged before processing 100 gal): $F = 4.762$, $v_1 = 3$, $v_2 = 20$; $P = 0.012$

Anova: (Runs in which all filters processed 100 gal): $F = 0.725$, $v_1 = 3$, $v_2 = 12$; $P = 0.556$

TABLE A4. ENTERIC VIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE TSM: COXSACKIEVIRUS TYPE A^a

Date	Balston				Cox				Carborundum-Cox				Millipore			
	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Virus Recov. (%)
4/15/77	100	128.9	6.9	5.4	100	128.9	4.6	3.6	100	128.9	10.3	8.0	98	126.2	2.3	1.8
4/19/77	100	33.3	2.3	6.9	107	35.5	2.3	6.5	101	33.6	5.7	17.0	100	33.3	3.5	10.5
5/3/77	100	62.8	9.1	14.5	100	62.9	28.8	45.8	100	62.8	18.3	29.1	100	62.8	16.1	25.6
5/5/77	100	37.5	11.5	30.7	100	37.5	15.0	40.0	100	37.3	21.9	58.7	100	37.5	8.0	21.3
5/10/77	100	115.7	41.8	36.1	100	115.7	37.3	32.2	100	115.7	47.7	41.2	100	115.7	50.8	43.9
5/12/77	100	227.1	18.4	8.1	100	227.1	60.1	26.5	100	227.1	92.2	40.6	100	227.1	48.5	21.4
6/1/77	100	38.3	5.9	15.4	100	38.3	10.6	27.7	100	38.3	7.0	18.3	100	38.3	24.0	62.7
6/3/77	100	84.3	5.8	6.9	100	84.3	25.4	30.1	82	69.2	28.7	41.5	100	84.3	26.5	31.4
Avg. ^b	100	91.0		15.5 (±9.8)	101	91.3		26.6 (±12.3)	98	89.1		31.8 (±14.0)	100	90.7		27.3 (±16.0)

^aLess-Thanⁿ values assumed 0% for calculations

^bAverage (95% confidence interval)

Anova: F = 1.536, v₁ = 3, v₂ = 28; P = 0.227

TABLE A5. ENTERIC VIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE TSM: BOVINE ENTEROVIRUS TYPE 1^a

Date	Balston				Cox				Carborundum-Cox				Millipore			
	Tap Water Vol. (gal)	Virus Input	Virus (PFU) Recov.	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input	Virus (PFU) Recov.	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input	Virus (PFU) Recov.	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input	Virus (PFU) Recov.	Virus Recov. (%)
6/30/77	100	238.5	75.4	31.6	102	242.8	176.7	72.8	100	238.5	53.5	22.4	100	238.5	62.9	26.4
8/1/77	100	38.4	6.8	17.2	100	39.4	11.2	28.4	100	39.4	5.7	14.5	100	39.4	9.2	23.4
8/19/77	110	223.7	36.6	17.2	103	209.0	11.5	5.5	100	204.1	34.2	16.8	100	205.1	18.2	8.9
8/24/77	100	38.7	12.7	32.8	100	38.7	23.0	59.4	100	38.7	40.3	104.1	100	38.7	19.4	50.1
8/26/77	100	120.6	3.4	2.8	101	120.9	11.5	9.5	100	120.6	11.4	9.4	100	120.6	8.0	6.6
8/31/77	100	47.2	1.2	2.5	100	47.1	4.5	9.6	101	47.5	7.0	14.7	100	47.1	5.8	12.3
9/2/77	101	112.1	4.6	4.1	100	111.7	9.3	8.3	100	111.8	21.0	18.8	81	90.0	1.2	1.3
9/20/77	99	56.1	9.9	17.6	100	56.5	3.7	6.5	100	56.5	11.3	20.0	100	56.5	6.1	10.8
Avg. ^b	101	109.5		15.7 (±10.1)	101	108.3		25.0 (±22.2)	100	107.1		27.1 (±24.9)	98	104.5		17.5 (±13.0)

^aLess-Than" values assumed 0%, 100: assumed for calculations

^bAverage (95% confidence interval)

Anova: F = 0.499, $v_1 = 3$, $v_2 = 28$; P = 0.696

TABLE A6. ENTERIC VIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE TSM: MINUTE VIRUS OF MICE^a

Date	Balston					Cox					Carbondene-Cox					Millipore				
	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)
		Input	Recov.			Input	Recov.			Input	Recov.			Input	Recov.			Input	Recov.	
10/11/77	101	71.8	22.1	30.8	109	79.7	6.0	7.5	100	59.0	20.9	35.4	100	60.5	15.9	26.3	100	128.5	-1.0	-0.8
10/18/77	100	108.3	-1.0	-0.9	100	105.6	1.2	1.1	100	142.7	9.3	6.5	100	128.5	-1.0	-0.8	100	119.8	-1.0	-0.8
10/28/77	100	101.0	-1.0	-1.0	100	98.5	-1.0	-1.0	100	131.5	-1.0	-0.8	100	128.5	-1.0	-0.8	100	119.8	-1.0	-0.8
11/2/77	100	72.7	4.0	5.5	100	70.9	1.3	1.8	100	94.7	-1.0	-1.0	100	86.3	3.8	4.4	100	57.3	-1.0	-1.7
4/12/78	100	54.9	-1.0	-1.8	100	57.3	-1.0	-1.7	100	52.1	-1.0	-1.9	100	57.3	-1.0	-1.7	100	33.7	-1.0	-3.0
4/20/78	101	37.1	-1.0	-2.7	100	34.1	-1.0	-2.9	100	34.6	-1.0	-2.9	100	33.7	-1.0	-3.0	100	33.7	-1.0	-3.0
Avg. ^b	100	74.3		6.0 (±12.9)	102	74.3		1.7 (±3.1)	100	85.8		7.0 (±14.9)	95	81.0		5.1 (±11.0)				

^aLess-Than" values assumed 0: for calculations

^bAverage (95% confidence interval)

Anova: $F = 0.266$, $v_1 = 3$, $v_2 = 20$; $p = 0.849$

TABLE A7. ENTERIC VIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE TSM: SIMIAN ADENOVIRUS 11^a

Date	Balston				Cox				Carborundum-Cox				Millipore			
	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input (PFU)	Virus Recov. (%)	Virus Recov. (%)
11/16/77	100	71.2	<1.1	<1.5	100	71.2	<1.1	<1.5	84	59.7	<1.1	<1.8	100	71.1	<1.1	<1.5
11/23/77	100	67.6	<1.1	<1.6	100	67.6	<1.1	<1.6	100	67.6	<1.1	<1.6	100	67.6	<1.1	<1.6
11/30/77	100	123.5	<1.1	<0.9	100	123.8	<1.1	<0.9	100	123.8	<1.1	<0.9	100	123.8	<1.1	<0.9
12/2/77	100	198.8	<1.1	<0.6	100	198.8	<1.1	<0.6	100	198.8	<1.1	<0.6	100	207.7	<1.1	<0.5
12/8/77	87	170.5	<1.1	<0.6	100	197.3	<1.1	<0.6	100	197.3	<1.1	<0.6	100	197.3	<1.1	<0.6
1/16/78	100	57.0	<1.2	<2.1	99	61.9	<1.2	<1.9	100	59.4	<1.1	<1.8	77	44.8	<1.2	<2.7
Avg. ^b	98	114.8		0	100	120.1		0	97	117.8		0	97	118.7		0

^aLess-Than" values assumed 0; for calculations

^bAverage

TABLE AB. ENTERIC VIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE TSM: ADENOVIRUS TYPE 5^a

Date	● Balston				Cox				Carborundum-Cox				Millipore			
	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal)	Virus (PFU)		Virus Recov. (%)
		Input	Recov.			Input	Recov.			Input	Recov.			Input	Recov.	
1/18/78	100	216.9	<1.3	-0.5	ND ^b				100	212.2	<1.6	-0.8	73	127.1	<1.0	-0.8
2/3/78	100	193.8	-1.1	-0.6	100	169.5	<1.1	<0.6	50	105.8	<1.1	<1.0	100	225.0	<1.1	-0.5
2/23/78	100	31.0	-1.1	-3.5	100	28.7	<1.1	<3.8	ND ^c				100	31.1	<1.1	-3.5
3/10/78	101	22.2	-1.2	-5.4	100	19.9	<1.1	-5.4	100	21.1	<1.2	-5.7	100	21.5	<1.1	-5.1
Avg. ^d	100	116.0		0	100	72.7		0	83	113.0		0	93	101.2		0

^a"Less-Than" values assumed 0: for calculations

^bContamination of cell cultures; no data

^cInput of <20 PFU/100 gal due to virus stock problems; no data

^dAverage

TABLE A9. ENTERIC VIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE TSM: RECOVERY TYPE 3^a

Date	Balston				Cox				Carborundum-Cox				Millipore			
	Tap Water Vol. (gal)	Virus Input	(PFU) Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input	(PFU) Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input	(PFU) Recov. (%)	Virus Recov. (%)	Tap Water Vol. (gal)	Virus Input	(PFU) Recov. (%)	Virus Recov. (%)
4/4/78	100	132.6	<2.3	<1.7	100	103.6	<2.1	<2.0	100	119.1	<2.4	<2.0	100	126.9	<2.1	<1.6
4/6/78	100	141.3	<2.4	<1.7	100	142.1	<2.4	<1.7	100	150.2	<2.4	<1.5	100	150.4	<2.1	<1.6
5/16/78	100	38.5	<1.2	<3.1	100	35.6	<1.2	<3.4	100	40.3	<1.2	<3.0	100	36.1	<2.6	<59.8
5/22/78	100	247.2	<2.0	<0.8	100	241.0	<2.0	<0.8	100	254.5	<1.9	<0.7	103	239.9	<2.1	<0.9
5/26/78	100	59.5	<1.1	<2.8	100	36.7	<1.2	<3.3	100	37.5	<1.2	<3.2	100	37.3	<1.2	<3.2
5/19/78	100	55.9	<1.2	<2.1	100	53.9	<1.2	<2.2	100	53.8	<1.1	<2.0	100	52.4	<1.1	<2.1
Avg. ^b	100	109.2		0	100	102.2		0	100	109.2		0	101	107.2		0

^aLess-Than" values assumed 0% for calculations

^bAverage

TABLE A10. VIRUS RECOVERIES FROM LARGE VOLUMES OF CHAPEL HILL TAP WATER WITH THE TSM:
SIMULTANEOUS EVALUATION OF FIVE ENTEROVIRUSES WITH COT FILTERS^a

SIMULTANEOUS EVALUATION OF FILTER ENTEROVIRUSES WITH COX FILTERS																								
Polio 1					Bovine Enterovirus 1					Coxsackie B3					Coxsackie A9					Echo 7				
Tap	Water				Tap	Water				Tap	Water				Tap	Water				Tap	Water			
Vol.	Vol.	Virus (PFU)	Virus Recov.		Vol.	Vol.	Virus (PFU)	Virus Recov.		Vol.	Vol.	Virus Recov.			Vol.	Vol.	Virus Recov.			Vol.	Vol.	Virus (PFU)	Virus Recov.	
Input	Input	Input	Input		Input	Input	Input	Input		Input	Input	Input	Input		Input	Input	Input	Input		Input	Input	Input	Input	
Recov.	Recov.	Recov.	Recov.		Recov.	Recov.	Recov.	Recov.		Recov.	Recov.	Recov.	Recov.		Recov.	Recov.	Recov.	Recov.		Recov.	Recov.	Recov.	Recov.	
(%)	(%)	(%)	(%)		(%)	(%)	(%)	(%)		(%)	(%)	(%)	(%)		(%)	(%)	(%)	(%)		(%)	(%)	(%)	(%)	
																</								

^aLess-Than" values assumed 0; for calculations

^bInput: -20 PFU/100 gal due to virus stock problems; no data

Shot included in trial

Assay failure: no data

Average (95% confidence interval)

Anova: $F = 1.984$, $v_1 = 4$, $v_2 = 50$; $p = 0.111$

TABLE A11. VIRUS RECOVERIES FROM LARGE VOLUMES OF CHAPEL HILL TAP WATER WITH THE TSM:
SIMULTANEOUS EVALUATION OF FIVE ENTERIC VIRUSES WITH COX FILTERS^a

Date	Polio 1				Reovirus 3				Adenovirus 5				SV-11			
	Tap Water Vol. (gal)	Virus (PFU)	Input	Recov.	Tap Water Vol. (gal)	Virus (PFU)	Input	Recov.	Tap Water Vol. (gal)	Virus (PFU)	Input	Recov.	Tap Water Vol. (gal)	Virus (PFU)	Input	Recov.
				(%)				(%)				(%)				(%)
1/26/78	100 ^b	87.2	6.0	6.9	ND ^b	61.6	<1.1	<1.8	100	66.1	<1.1	<1.7	ND ^c	<1.1	57.0	<1.9
2/17/78	100	143.0	<2.0	<1.4	100	87.8	<1.1	<1.2	100	125.0	<1.1	<0.9	100	<1.1	107.0	<1.0
5/4/78	100	53.2	6.7	12.6	ND ^b	136.6	<1.1	<0.8	100	153.9	<1.1	<0.7	100	<1.1	32.0	<3.4
6/7/78	100	89.0	<2.0	<2.2	121	377.0	<1.1	<0.3	121	147.9	<1.1	<0.2	100	<1.1	60.7	<1.8
6/21/78	100	21.2	<2.3	<10.8	ND ^b	377.0	<1.1	<0.3	115	637.0	<1.1	<1.3	115	<1.1	516.0	<0.2
7/6/78	121	138.6	9.4	6.8	100	973.7	<1.2	<0.1	100	247.2	<1.1	<0.5	121	<1.1	1350.0	<0.1
7/20/78	121	67.3	4.6	6.8	ND ^b	319.2	2.1	0.6	100	375.5	<1.1	<0.4	100	<1.1	182.7	<0.6
10/5/78	121	22.1	2.3	10.4	ND ^b	319.2	2.1	0.6	100	256.3	<1.1	<1.2	100	<1.1	105.8	<1.0
11/2/78	121	30.3	20.0	66.0	121	319.2	2.1	0.6	121	91.5	<1.1	<1.2	100	<1.1	91.5	<1.1
12/6/78	100	120.0	<1.5	<1.2	121	319.2	2.1	0.6	121	91.5	<1.1	<1.2	100	<1.1	91.5	<1.1
Avg. ^d	108	77.2		11.0 (±14.2)	111	326.0		0.1 (±0.2)	105	201.2		0	104	282.5		0

^aLess-Than" values assumed 0% for calculations

^bInput <20 PFU/100 gal due to virus stock problems; no data

^cAssay failure; no data

^dAverage (95% confidence interval)

TABLE A12. VIRUS RECOVERIES FROM LARGE VOLUMES OF CHAPEL HILL TAP WATER
WITH THE TSM: SIMULTANEOUS EVALUATION OF POLIOVIRUS AND MMV^a

Date	Polio			MMV		
	Tap Water Vol. (gal)	Virus (PFU) Input	Recov. (%)	Tap Water Vol. (gal)	Virus (PFU) Input	Virus Recov. (%)
					Recov.	
1/26/78	100	87.2	6.0	100	118.7	<2.4
2/17/78	ND ^b			100	36.5	<2.0
4/14/78	100	143.0	<2.0	100	34.0	<2.0
6/9/78	100	147.6	7.4	100	21.8	<2.0
8/1/78	103	67.6	<2.0	ND ^b		
9/15/78	121	15.6	2.4	100	136.8	2.2
10/3/78	100	87.6	4.6	121	172.6	<2.0
10/26/78	ND ^c			121	2253.0	87.1
11/2/78	121	30.3	20.0	121	451.1	91.7
11/6/78	ND ^c			121	611.8	28.3
12/4/78	100	439.6	<2.0	100	232.7	7.7
12/6/78	100	120.0	<1.5	100	25.1	<2.3
Avg. ^d	105	126.5	10.9 (±16.3)	104	372.2	3.1 (±4.0)

^a"Less-Than" values assumed 0% for calculations

^bInput <20 PFU/100 gal due to virus stock problems; no data

^cAssay failure; no data

^dAverage (95% confidence interval)

Two-sample t-test (polio vs. MMV): $t = 1.180$, $df = 18$; $P = 0.127$

TABLE A13. POLIOVIRUS RECOVERIES FROM CHAPEL HILL TAP WATER WITH THE TSM^a

Date	Ballston				Cox				Carborundum-Cox				Hillipore			
	Tap Water Vol. (gal.)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal.)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal.)	Virus (PFU)		Virus Recov. (%)	Tap Water Vol. (gal.)	Virus (PFU)		Virus Recov. (%)
		Input	Recov.			Input	Recov.			Input	Recov.			Input	Recov.	
10/13/77	100	37.3	<1.0	<2.7	100	45.4	<1.0	<2.2	100	38.2	1.2	3.1	100	50.4	<1.0	<2.0
11/4/77	100	98.4	<1.0	<1.0	100	75.5	<1.0	<1.3	100	88.7	8.1	9.0	100	73.7	<1.0	<1.4
12/6/77	100	62.7	19.4	30.9	104	69.3	17.1	24.7	100	83.2	24.0	46.9	100	65.6	4.6	7.0
1/20/78	100	102.0	<1.1	<1.1	100	53.3	<1.1	<1.2	98	100.5	<1.1	<1.1	57	50.4	<1.1	<2.2
3/23/78	100	58.2	<1.1	<1.9	100	52.6	<1.1	<2.7	100	134.1	<1.0	<2.9	100	41.0	<1.1	<2.7
3/31/78	100	140.6	<1.2	<0.8	100	150.1	<1.1	<0.7	100	147.1	1.2	0.8	100	141.7	<1.1	<0.6
4/24/78	100	60.8	4.4	2.2	100	59.9	5.6	9.3	100	57.5	1.1	2.3	100	42.7	5.9	9.4
5/12/78	100	50.2	1.1	2.2	100	52.2	<1.1	<2.5	100	51.0	1.1	2.5	100	51.7	<1.1	<2.1
5/30/78	100	47.3	<1.1	<2.3	100	43.6	<1.1	<1.8	100	43.6	2.4	3.7	100	45.6	<1.1	<2.4
6/15/78	100	60.5	4.6	4.8	100	62.3	<1.1	2.3	100	63.0	<1.1	<1.7	100	61.5	<1.1	<1.8
6/29/78	100	96.7	2.4	3.0	103	93.9	2.2	7.4	100	85.7	6.8	7.9	100	100.4	13.8	13.7
7/10/78	101	79.6	<1.1	<0.4	100	84.4	6.3	<1.0	100	72.9	2.4	3.3	100	75.2	1.0	1.3
10/11/78	100	278.5	<1.1	0.6	100	284.1	21.9	<0.4	100	278.9	1.3	0.5	100	308.1	<1.3	<0.4
11/8/78	100	605.2	3.4	0.6	100	584.8	21.9	3.7	100	544.0	13.9	2.6	100	554.2	3.5	0.6
11/14/78	100	528.0	10.8	2.0	100	299.5	10.4	3.5	100	477.0	23.4	4.9	100	552.0	13.6	2.5
Avg. ^b	100	153.7		3.4 (±4.4)	101	136.7		3.4 (±3.6)	100	142.3		5.9 (±6.5)	97	148.9		2.3 (±2.2)

^a"Less-Than" values assumed 0% for calculations^bAverage (95% confidence interval)Anova: F = 0.546, v₁ = 3, v₂ = 56; P = 0.653

TABLE A14. RECOVERIES OF ENTEROVIRUSES FROM GAC-TREATED AND UNTREATED TAP WATER: SIMULTANEOUS EXPERIMENTS USING 12 L WATER VOLUMES AND 47-MM DIAMETER COX FILTERS IN SINGLE STEP CONCENTRATION PROCEDURE^a

Date	Polio 1						Coxsackie B-3						Coxsackie A-9					
	Untreated			GAC-Treated			Untreated			GAC-Treated			Untreated			GAC-Treated		
	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.
9/22/78	37.1	21.0	56.6	ND ^b	ND ^c	ND ^c	ND ^b	74.0	6.6	8.9	6.7	9.0	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
10/20/78	55.0	46.6	84.7	ND ^b	ND ^c	ND ^c	105.4	3.3	3.1	3.0	3.3	3.0	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
11/3/78	43.9	16.4	37.4	ND ^b	ND ^c	ND ^c	316.5	3.3	3.1	3.0	7.6	2.5	1660.0	36.3	2.2	202.1	12.2	6.0
11/17/78	500.3	76.7	15.3	ND ^b	ND ^c	ND ^c	344.0	3.1	3.1	0.9	3.1	0.8	1660.0	23.6	1.0	13.6	1.4	0.1
12/7/78	293.3	46.9	16.0	ND ^b	ND ^c	ND ^c	210.0	3.1	3.1	1.5	8.1	3.8	963.3	23.6	2.4	127.8	13.3	1.4
12/21/78	237.0	13.4	5.6	ND ^b	ND ^c	ND ^c	210.6	3.1	3.1	3.1	3.1	3.8	1197.8	1.9	0.2	9.0	9.0	0.0
Avg. ^e	194.4	26.3	13.5	ND ^b	ND ^c	ND ^c	210.6	3.1	3.1	3.1	3.1	3.8	1197.8	1.9	0.2	9.0	9.0	0.0

Date	Polio 1						Bovine Enterovirus 1						Echo 7					
	Untreated			GAC-Treated			Untreated			GAC-Treated			Untreated			GAC-Treated		
	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.	Input (PFU)	Rec.	%Rec.
9/14/78	ND ^b	8.6	39.4	7.0	32.1	45.9	165.8	11.7	7.0	40.5	24.4	14.5	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
9/26/78	21.8	2.3	10.5	15.8	22.2	102.3	132.0	36.4	27.6	57.4	43.5	24.4	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
10/20/78	79.3	23.3	29.4	16.7	12.4	74.3	220.0	9.0	4.1	74.3	33.8	15.5	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
10/27/78	39.3	53.3	135.6	16.7	16.8	101.2	70.6	4.4	6.2	13.1	18.5	79.5	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
11/9/78	454.5	63.3	13.9	26.7	16.8	62.9	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
12/1/78	264.0	2.4	0.9	22.4	10.0	38.2	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
12/15/78	304.9	54.6	17.9	30.5	10.0	32.8	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
12/28/78	106.0	10.2	9.6	23.4	22.1	20.8	42.8	15.1	35.3	21.3	49.8	32.2	ND ^b	ND ^c	ND ^c	ND ^b	ND ^c	ND ^c
Avg.	180.3	20.4	11.3	20.8	20.8	20.8	110.4	17.3	17.3	17.3	32.2	32.2	264.7	34.2	34.2	42.3	42.3	42.3

^aLess-Than values assumed 0%, >100% assumed 100% for calculations

^bInput <20 PFU/12 l due to virus stock problems; no data

^cSpot included in experiment

^dAssay failure; no data

^eAverage (95% confidence interval)

Table 14 (cont.)

Anova (untreated tap water): $F = 2.222$, $v_1 = 4$, $v_2 = 27$; $P = 0.093$

Anova: (GAC-treated tap water): $F = 3.691$, $v_1 = 4$, $v_2 = 21$; $P = 0.020$

Two-sample t-test Polio (CB3/CA9) vs. Polio (BE-1, E7), Untrt. : $t = 0.420$,
 $df = 11$; $P = 0.341$

Paired t-test

- 1) Polio (untrt) vs. Polio (GAC): $t = -0.094$, $df = 6$; $P = 0.464$
- 2) CB3 (untrt) vs. CB3 (GAC): $t = -1.643$, $df = 4$; $P = 0.088$
- 3) CA9 (untrt) vs. CA9 (GAC): $t = -2.113$, $df = 2$; $P = 0.084$
- 4) BE-1 (untrt) vs. BE-1 (GAC): $t = -3.842$, $df = 5$; $P = 0.006$
- 5) E7 (untrt) vs. E7 (GAC): $t = -1.252$, $df = 4$; $P = 0.139$

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